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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/11, 9/00, A61K 48/00, C12N 5/10

A2

(11) International Publication Number: WO 95/31541

(43) International Publication Date: 23 November 1995 (23.11.95)

(21) International Application Number: PCT/US95/06368

(22) International Filing Date: 18 May 1995 (18.05.95)

(30) Priority Data:

08/245,466	18 May 1994 (18.05.94)	US
08/373,124	13 January 1995 (13.01.95)	US

(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

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(54) Title: METHODS AND COMPOSITIONS FOR TREATMENT OF RESTENOSIS AND CANCER USING RIBOZYMES

(57) Abstract

An enzymatic nucleic acid molecule which cleaves *c-myb* RNA, wherein the binding arms of said nucleic acid contain sequences complementary to the sequences defined in Tables II, XII-XXIV.

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U.S. Serial No.: 09/887,880
Filed: June 22, 2001 **(Exhibit A)**

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DESCRIPTION

Methods and Compositions for Treatment of Restenosis and Cancer Using Ribozymes

Background Of The Invention

The present invention concerns therapeutic compositions and methods for the treatment of restenosis and cancer.

The following is a brief description of the physiology, cellular pathology and treatment of restenosis. The discussion is not meant to be complete and is provided only for understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

Coronary angioplasty is one of the major surgical treatments for heart disease. Its use has been accelerating rapidly; over 450,000 procedures are performed in the U.S. annually. The short term success rate of angioplasty is 80 to 90%. However, in spite of a number of technical improvements in the procedure, post-operative occlusions of the arteries, or restenosis, still occur. Thirty-five to forty-five percent of patients who have undergone a single vessel angioplasty develop clinically significant restenosis within 6 months of the procedure. The rate of restenosis is even higher (50 to 60%) in patients who have undergone multivessel angioplasty (Califf, R. M., et al., 1990, in *Textbook of Interventional Cardiology.*, E.J. Topol, ed., W. B. Saunders, Philadelphia, pp 363-394.).

Histopathological studies have shown that restenosis after angioplasty is characterized by migration of medial smooth muscle cells to the intima and a striking hyperproliferative response of these neointimal cells (Garratt, K. N., et al., 1991, *J. Am. Coll. Cardio.*, 17, 442-428; Austin, G. E., et al., 1985, *J . Am. Coll. Cardiol*., 6, 369-375). Smooth muscle cell proliferation could be an overly robust response to injury. Alternatively, the

intimal smooth muscle cells within atherosclerotic lesions are already in an activated or "synthetic" state (Sjolund, M., et al., 1988, *J. Cell. Biol.*, 106, 403-413 and thus may be poised to proliferate. One recent study demonstrated a positive correlation between the presence of activated smooth muscle cells in coronary lesions and the extent of subsequent luminal narrowing after atherectomy (Simons, M., et al., 1993, *New Engl. J. Med.*, 328, 608-613). In any case, slowing smooth muscle cell proliferation after angioplasty could prevent intimal thickening and restenosis.

The presently preferred therapeutic treatment for restenosis is the use of streptokinase, urokinase or other thrombolytic compounds, such as fish oil, anticoagulants, ACE (angiotensin converting enzyme) inhibitors, aspirin and cholesterol lowering compounds. Alternative treatment includes the surgical incorporation of endoluminal stents. The occurrence of pharmacologic side-effects (particularly bleeding disorders associated with anti-coagulants and platelet inhibitors) is an issue with current therapies. Popoma, J. J., et al., report that the current therapies have not significantly impacted the rates of restenosis occurrence. (*Circulation*, 84, 1426-1436, 1991).

Recently, the results of a clinical trial of the efficacy of an anti-platelet therapy have been reported. Patients undergoing coronary angioplasty were given a single bolus injection followed by a 12 hour infusion of an antibody directed against the platelet adhesion molecule, gpIIb/gpIIIa. After six months, patients with the treatment showed a 23% reduction in the occurrence of restenosis than patients receiving placebo (27 vs. 35%; p=0.001).

A number of growth factors have been shown to induce smooth muscle cell proliferation. *In vitro*, platelet-derived growth factor (PDGF) is a potent smooth muscle cell mitogen (Ross, R., et al., 1974, *Proc. Natl. Acad. Sci. USA*, 71, 1207-1210) and a smooth muscle cell chemo-

attractant (Grotendorst, G., et al., 1982, *Proc. Natl. Acad. Sci. USA*, 71, 3669-3672.). *In vivo*, when PDGF is expressed ectopically in porcine arteries, it induces intimal hyperplasia (Nabel, E. B., et al., 1993, *J. Clin. Invest.*, 91, 1822-1829). Furthermore, antibodies to PDGF have been shown to reduce intimal thickening after arterial injury (Ferns, G. A. A., et al., 1991, *Science*, 253, 1129-1132). Analysis of ^{3}H-thymidine incorporation in the lesions indicates that the anti-PDGF antibodies primarily inhibit smooth muscle cell migration.

Basic fibroblast growth factor (bFGF) is another smooth muscle cell mitogen *in vitro* (Klagsbrun, M. and Edelman, E. R., 1989, *Arteriosclerosis*, 9, 269-278). In a rat model, anti-bFGF antibodies inhibit the proliferation of medial smooth muscle cells 24 to 48 hours after balloon catheter injury (Lidner, V. and Reidy, M. A., 1991, *Proc. Natl. Acad. Sci. USA*, 88, 3739-3743). In addition to bFGF, heparin binding epidermal growth factor (HB-EGF) (Higashiyama, S., et al., 1991, *Science*, 251, 936-939.), insulin-like growth factor I (IGF-I) (Banskota, N. K., et al., 1989, *Molec. Endocrinol.*, 3, 1183-1190) and endothelin (Komuro, I., et al., 1988, *FEBS Letters*, 238, 249-252) have been shown to induce smooth muscle cell proliferation. A number of other factors (such as interleukin-1 and tumor necrosis factor-α) may indirectly affect smooth muscle cell proliferation by inducing the expression of PDGF (Hajjar, K. A., et al., 1987, *J. Exp. Med.*, 166, 235-245; Raines, E. W., et al., 1989, *Science*, 243, 393-396).

When whole serum is added to serum-starved smooth muscle cells *in vitro*, the oncogenes, c-*myc*, c-*fos*, and c-*myb*, are induced (Kindy, M. S. and Sonenshein, G. E., 1986, *J. Biol. Chem.*, 261, 12865-12868; Brown, K. E., et al., 1992, *J. Biol. Chem.*, 267, 4625-4630) and cell proliferation ensues. Blocking c-*myb* with an antisense oligonucleotide prevents cells from entering S phase (Brown, K. E., et al., 1992, *J. Biol. Chem.*, 267, 4625-

4630.). Thus, c-*myb* is required for the G_1 to S transition after stimulation by the multitude of growth factors present in serum. *In vivo,* a c-*myb* antisense oligonucleotide inhibits restenosis when applied to rat arteries after balloon angioplasty (Simons, M., et al., 1992, *Nature*, 359, 67-70). Similarly, an antisense oligonucleotide directed against mRNA of the oncogene *c-myc* was shown to inhibit human smooth muscle cell proliferation (Shi, Y., et al., 1993, *Circulation*, 88, 1190-5) and migration (Biro, S., et al., 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 654-8).

Ohno et al., 1994 *Science* 265, 781, have shown that a combination of viral thymidine kinase enzyme expression (gene therapy) and treatment with anti-viral drug ganciclovir inhibits smooth muscle cell proliferation in pigs, following baloon angioplasty.

Epstein et al., "Inhibition of non-transformed cell proliferation using antisense oligonucleotides," NTIS publication 1992 discusses use of antisense oligonucleotides to c-*myc,* PCNA or cyclin B. Fung et al., PCT WO91/15580, describes gene therapy for cell proliferative disease and mentions administration of a ribozyme construct against a PGR element. Mention is made of inactivation of c-*myb*. Rosenberg et al., WO93/08845, Calabretta et al., WO92/20348 and Gewirtz WO93/09789 concern c-*myb* antisense oligonucleotides for treatment of melanoma or colorectal cancer, and administration locally. Sytkowski, PCT WO 93/02654, describe the uses of antisense oligonucleotides to inhibit *c-myb* gene expression in red blood cells to stimulate hemoglobin synthesis.

Nabel and Nabel, U. S. Patent No. 5, 328, 470, describe a method for the treatment of diseases by delivering therapeutic reagents directly to the sites of disease. They state that-

> "...Method is based on the delivery of proteins by catheterization to discrete blood vessel segments using genetically modified or normal cells

> or other vector systems... In addition, catalytic RNAs, called ribozymes, can specifically degrade RNA sequences.... The requirements for a successful RNA cleavage include a hammerhead structure with conserved RNA sequence at the region flanking this structure..... any GUG sequence within the RNA transcript can serve as a target for degradation by the ribozyme.... gene transfer using vectors expressing such proteins as tPA for the treatment of thrombosis and restenosis, angiogenesis or growth factors for the purpose of revascularization..."

Sullivan and Draper, International PCT publication WO 94/02595 describe the use of ribozymes against *c-myb* RNA to treat stenosis.

Summary Of The Invention

This invention relates to ribozymes, or enzymatic RNA molecules, directed to cleave mRNA species that are required for cellular growth responses. In particular, applicant describes the selection and function of ribozymes capable of cleaving RNA encoded by the oncogene, *c-myb*. Such ribozymes may be used to inhibit the hyperproliferation of smooth muscle cells in restenosis and of tumor cells in numerous cancers. To block restenosis, a target molecule required for the induction of smooth muscle cell proliferation by a number of different growth factors is preferred. To this end *c-myc*, *c-fos*, and *c-myb* are useful targets in this invention.

Other transcription factors involved in the response to growth and proliferation signals include NF-κB, *oct-1* and SRF. NF-κB protein activates cellular transcription and induces increases in cellular synthetic pathways. In a resting cell, this protein is found in the cytoplasm, complexed with its inhibitor, I-κB. Upon phosphorylation of the I-κB molecule, the complex dissociates and NF-κB is released for transport to the nucleus, where it binds DNA

and induces transcriptional activity in (NF-κB)-responsive genes. One of the (NF-κB)-responsive genes is the NF-κB gene itself. Thus, release of the NF-κB protein from the inhibitory complex results in a cascade of gene expression which is auto-induced. Early inhibition of NF-κB can reduce expression of a number of genes required for growth and proliferation, such as *c-myb*.

Two other transcription factors, *oct-1* and serum response factor (SRF) have been shown to be expressed selectively in dividing cells. Both *oct-1* and SRF are expressed ubiquitously in cultured cells, including smooth muscle cells. However, R. Majack and his colleagues have recently shown that these transcription factors are not expressed by the smooth muscle cells in intact vessels. Both *oct-1* and SRF are rapidly expressed upon dispersal of tissue into single cell suspensions. Thus, these transcription factors are thought to be regulated by their interactions with the extracellular matrix (Weiser, M. C. M., et al., 1994, J. Cell. Biochem., S18A, 282; Belknap, J. K., et al., 1994, J. Cell. Biochem., S18A, 277). Upon injury during angioplasty, the expression of *oct-1* and SRF may be enhanced, leading to increased smooth muscle cell proliferation. Treatment with ribozymes that block the expression of these transcription factors can alleviate the smooth muscle cell proliferation associated with restenosis.

While some of the above mentioned studies demonstrated that antisense oligonucleotides can efficiently reduce the expression of factors required for smooth muscle cell proliferation, enzymatic RNAs, or ribozymes have yet to be demonstrated to inhibit smooth muscle cell proliferation. Such ribozymes, with their catalytic activity and increased site specificity (as described below), represent more potent and safe therapeutic molecules than antisense oligonucleotides. In the present invention, ribozymes that cleave *c-myb* mRNA are described. Moreover, applicant shows that these ribozymes are able to

inhibit smooth muscle cell proliferation and that the catalytic activity of the ribozymes is required for their inhibitory effect. From those of ordinary skill in the art, it is clear from the examples described, that other ribozymes that cleave target mRNAs required for smooth muscle cell proliferation may be readily designed and are within the invention.

By "inhibit" is meant that the activity of *c-myb* or level of mRNAs encoded by *c-myb* is reduced below that observed in the absence of the nucleic acid, particularly, inhibition with ribozymes and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

By "enzymatic nucleic acid molecule" it is meant a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to *c-myb* is meant to include those naturally occurring RNA molecules associated with restenosis and cancer in various animals, including human, rat and pig. Such a molecule will generally contain some ribonucleotides, but the other nucleotides may be substituted at the 2'-hydroxyl position and in other locations with other moeities as discussed below.

By "complementarity" is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example, Hoogsteen type) of base-paired interactions.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf, T. M., et al., 1992, _Proc. Natl. Acad. Sci. USA_, 89, 7305-7309). Thus, the specificity of

action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, *Aids Research and Human Retroviruses* 8, 183, of hairpin motifs by Hampel *et al.*, EP0360257, Hampel and Tritz, 1989 *Biochemistry* 28, 4929, and Hampel *et al.*, 1990 *Nucleic Acids Res.* 18, 299, and an example of the hepatitis delta virus motif is described by Perrotta and Been, 1992 *Biochemistry* 31, 16; of the RNaseP motif by Guerrier-Takada *et al.*, 1983 *Cell* 35, 849, *Neurospora* VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 *Cell* 61, 685-696; Saville and Collins, 1991 *Proc. Natl. Acad. Sci. USA* 88, 8826-8830; Collins and Olive, 1993 *Biochemistry* 32, 2795-2799) and of the Group I intron by Cech *et al.*, U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding c-myb proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered

exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA/RNA vectors that are delivered to specific cells.

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small enzymatic nucleic acid motifs (*e.g.*, of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. However, these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet et al., 1992 *Antisense Res. Dev.*, 2, 3-15; Dropulic et al., 1992 *J. Virol*, 66, 1432-41; Weerasinghe et al., 1991 *J. Virol*, 65, 5531-4; Ojwang *et al.*, 1992 *Proc. Natl. Acad. Sci. USA* 89, 10802-6; Chen *et al.*, 1992 *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science* 247, 1222-1225). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their totality by reference herein; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem. 269, 25856).

Thus, in a first aspect, the invention features ribozymes that inhibit cell proliferation. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or

hairpin motif. Upon binding, the ribozymes cleave the target mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, cell proliferation is inhibited.

In a preferred embodiment, the enzymatic RNA molecules cleave *c-myb* mRNA and inhibit smooth muscle cell proliferation. Such ribozymes are useful for the prevention of restenosis after coronary angioplasty. Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to smooth muscle cells. The RNA or RNA complexes can be locally administered to relevant tissues through the use of a catheter, infusion pump or stent, with or without their incorporation in biopolymers. The ribozymes, similarly delivered, also are useful for inhibiting proliferation of certain cancers associated with elevated levels of the *c-myb* oncogene, particularly leukemias, neuroblastomas, and lung, colon, and breast carcinomas. Using the methods described herein, other enzymatic RNA molecules that cleave *c-myb*, *c-myc*, *oct-1*, SRF, NF-κB, PDGF receptor, bFGF receptor, angiotensin II, and endothelium-derived relaxing factor and thereby inhibit smooth muscle cell proliferation and/or tumor cell proliferation may be derived and used as described above. Specific examples are provided below in the Tables.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of *c-myb* activity in a cell or tissue. By "related" is meant that the inhibition of *c-myb* mRNAs and thus reduction in the level of protein activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection,

infusion pump or stent, with or without their incorporation in biopolymers.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit *c-myb* activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alpha-virus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells explanted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in the tables II, XII-XXIV. Examples of such ribozymes are shown as Seq. I.D. Nos. 101-129 (table III) and in tables XII-XXIV. By complementary is thus meant that the binding arms are able to cause cleavage of a human or mouse or rat or porcine mRNA target. Examples of such ribozymes consist essentially of sequences defined in tables III, XII-XXIV. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind *c-myb* mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit cell proliferation are expressed from transcription units inserted into DNA, RNA, or viral vectors. Preferably, the recombinant vectors capable of expressing the ribozymes are locally delivered as described above, and transiently persist in smooth muscle cells. Once expressed, the ribozymes cleave their target mRNAs and prevent proliferation of their host cells. The recombinant vectors are preferably DNA plasmids or adenovirus vectors. However, other mammalian cell vectors that direct the expression of RNA may be used for this purpose.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description Of The Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be ≥ 2 base-pair long.

Figure 2a is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, *Nature* , 334, 585-591) into two portions; and Figure 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or

more bases (preferably 3 - 20 bases, *i.e.*, m is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.*, r is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (*i.e.*, o and p is each independently from 0 to any number, *e.g.*, 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "_____" refers to a covalent bond.

Figure 4 is a representation of the general structure of the hepatitis delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a schematic representation of an RNAseH accessibility assay. Specifically, the left side of Figure 6 is a diagram of complementary DNA oligonucleotides bound to accessible sites on the target RNA. Complementary DNA oligonucleotides are represented by broad lines labeled A, B, and C. Target RNA is represented by the thin, twisted line. The right side of

Figure 6 is a schematic of a gel separation of uncut target RNA from a cleaved target RNA. Detection of target RNA is by autoradiography of body-labeled, T7 transcript. The bands common to each lane represent uncleaved target RNA; the bands unique to each lane represent the cleaved products.

Figure 7 is a graph of the results of an RNAseH accessibility assay of murine *c-myb* RNA. On the abscissa is the sequence number of the DNA oligonucleotide that is homologous to the ribozyme target site. The ordinate represents the percentage of the intact transcript that was cleaved by RNAse H.

Figure 8 is a graph of the outcome of an RNAseH accessibility assay of human *c-myb* mRNA. The graphs are labeled as in Figure 7.

Figure 9 shows the effect of chemical modifications on the catalytic activity of hammerhead ribozyme targeted to *c-myb* site 575. A) diagrammatic representation of 575 hammerhead ribozyme•substrate complex. 2'-O-methyl ribozyme represents a hammerhead (HH) ribozyme containing 2'-O-methyl substitutions at five nucleotides in the 5' and 3' termini. 2'-O-methyl P=S ribozyme represents a hammerhead (HH) ribozyme containing 2'-O-methyl and phosphorothioate substitutions at five nucleotides in the 5' and 3' termini. 2'-C-allyl iT ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl group substitutions, wherein 30 nucleotides contain 2'-O-methyl substitutions and one nucleotide (U_4) contains 2'-C-allyl substitution. Additionally, 3' end of this ribozyme contains a 3'-3' linked inverted T. 2'-C-allyl P=S ribozyme is similar to 2'-C-allyl iT ribozyme with the following changes: five nucleotides at the 5' and 3' termini contain phosphorothioate substitutions and the ribozyme lacks the 3'-end inverted T modification. B) shows the ability of ribozymes described in Fig. 9A to inhibit smooth muscle cell proliferation.

Figure 10 shows the effect of 2'-C-allyl P=S 575 HH ribozyme concentration on smooth muscle cell proliferation. A plot of percent inhibition of smooth muscle cell proliferation (normalized to the effect of a catalytically inactive ribozyme) as a function of ribozyme concentration is shown.

Figure 11 shows a comparison of the effects of 2'-C-allyl P=S 575 HH ribozyme and phosphorothioate antisense DNA on the proliferation of smooth muscle cells.

Figure 12 shows the inhibition of smooth muscle cell proliferation catalyzed by 2'-C-allyl P=S HH ribozymes targeted to sites 549, 575, and 1533 within *c-myb* mRNA.

Figure 13 shows the effect of phosphorthioate substitutions on the catalytic activity of 2'-C-allyl 575 HH ribozyme. A) diagrammatic representation of 575 hammerhead ribozyme•substrate complex. 10 P=S 5' and 3' ribozyme is identical to the 2'-C-allyl P=S ribozyme described in Fig. 9. 5 P=S 3' ribozyme is same as 10 P=S 5' and 3' ribozyme, with the exception that only five nucleotides at the 3' termini contain phosphorothioate substitutions. 5 P=S Loop ribozyme is similar to 2'-C-allyl iT described in Fig. 9, with the exception that five nucleotides within loop II of this ribozyme contain phosphorothioate substitutions. 5 P=S 5' ribozyme is same as 10 P=S 5' and 3' ribozyme, with the exception that only five nucleotides at the 5' termini contain phosphorothioate substitutions. Additionally, this ribozyme contains a 3'-3' linked inverted T at its 3' end. B) shows the ability of ribozymes described in Fig. 13A to inhibit smooth muscle cell proliferation.

Figure 14 shows the minimum number of phosphorothioate substitutions required at the 5' termini of 575 HH ribozyme to achieve efficient inhibition of smooth muscle cell proliferation.

Figure 15 shows the effect of varying the length of substrate binding arm of 575 HH ribozyme on the inhibition of smooth muscle cell proliferation.

Figure 16 shows the effect of various chemical modifications, at U_4 and/or U_7 positions within 575 HH ribozyme core, on the ability of the ribozyme to inhibit smooth muscle cell proliferation.

Figure 17 shows the inhibition of pig smooth muscle cell proliferation by active *c-myb* 575 HH ribozyme.

Figure 18 shows the inhibition of human smooth muscle cell proliferation by active *c-myb* 575 HH ribozyme.

Figure 19 shows ribozyme-mediated inhibition of *c-myb* expression and cell proliferation.

Figure 20 is digrammatic representation of an optimal *c-myb* HH ribozyme that can be used to treat diseases like restenosis.

Figure 21 shows the inhibition of Rat smooth muscle cells by 2-5A containing nucleic acids.

<u>Target sites</u>

Targets for useful ribozymes can be determined as disclosed in Draper et al <u>supra,</u> Sullivan *et al.*, <u>supra</u>, as well as by Draper et al., "Method and reagent for treatment of arthritic conditions PCT No. PCT/US94/13129, U.S.S.N. 08/152,487, filed 11/12/93, and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be optimized and delivered as described therein. While specific examples to mouse RNA are provided, those in the art will recognize that equivalent human RNA targets can be used as described below. Thus, the same target may be used, but binding arms suitable for targetting human RNA sequences are present in the ribozyme. Such targets may also be selected as described below.

The sequence of human, pig and murine *c-myb* mRNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables II and XII-XXIV (All sequences are 5' to 3' in the tables) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. While murine, pig and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, murine and pig targeted ribozymes may be useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Hammerhead or hairpin ribozymes were designed that could bind and were individually analyzed by computer folding (Jaeger *et al.*, 1989 *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

The sequences of the ribozymes that are chemically synthesized, useful in this study, are shown in Table III and XII-XXIV. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Table III (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of

hairpin ribozymes listed in Table III, XIII, XVI, XIX, XX, XXIII, XXIV (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The ribozyme sequences listed in Table III and XII-XXIV may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described in this application. These include altering the length of the ribozyme binding arms (stems I and III, see Figure 2c), or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken et al., 1991 *Science* 253, 314; Usman and Cedergren, 1992 *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Usman, N. *et al.* US Patent Application 07/829,729, and Sproat, US Patent No. 5, 334, 711 which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules, modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements. (All these publications are hereby incorporated by reference herein.)

Sullivan, *et al.*, *supra*, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes

may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan *et al.*, supra and Draper *et al.*, *supra* which have been incorporated by reference herein.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA or RNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993 *Nucleic Acids Res.*, 21, 2867-72; Lieber et al., 1993 *Methods Enzymol.*, 217, 47-66; Zhou et al., 1990 *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 *Antisense Res. Dev.*, 2, 3-15; Ojwang et al., 1992 *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992 *Nucleic Acids Res.*, 20, 4581-9; Yu et al., 1993 *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992 *EMBO J.* 11, 4411-8; Lisziewicz *et al.*, 1993 *Proc. Natl. Acad. Sci.*

U. S. A., 90, 8000-4). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves mRNAs encoded by *c-myb* is inserted into a plasmid DNA vector or an adenovirus or adeno-associated virus DNA viral vector or a retroviral RNA vector. Viral vectors have been used to transfer genes and lead to either transient or long term gene expression (Zabner et al., 1993 Cell 75, 207; Carter, 1992 Curr. Opi. Biotech. 3, 533). The adenovirus vector is delivered as recombinant adenoviral particles. The DNA may be delivered alone or complexed with vehicles (as described for RNA above). The recombinant adenovirus or AAV particles are locally administered to the site of treatment, e.g., through incubation or inhalation *in vivo* or by direct application to cells or tissues *ex vivo*.

In another preferred embodiment, the ribozyme is administered to the site of *c-myb* expression (e.g., smooth muscle cells) in an appropriate liposomal vesicle.

Examples

Ability Of Exogenously-Delivered Ribozymes Directed Against *c-myb* To Inhibit Vascular Smooth Muscle Cell Proliferation

The following examples demonstrate the selection of ribozymes that cleave *c-myb* mRNA. The methods described herein represent a scheme by which ribozymes may be derived that cleave other mRNA targets required for cell division. Also provided is a description of how such ribozymes may be delivered to smooth muscle cells. The examples demonstrate that upon delivery, the ribozymes inhibit cell proliferation in culture. Moreover, no

inhibition is observed if mutated ribozymes that are catalytically inactive are applied to the cells. Thus, inhibition requires the catalytic activity of the ribozymes. The cell division assay used represents a model system for smooth muscle cell hyperproliferation in restenotic lesions.

Example 1: Identification of Potential Ribozyme Cleavage Sites in Human *c-myb* mRNA

The sequence of human c-*myb* mRNA was screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and contained potential hammerhead ribozyme cleavage sites were identified. These sites are shown in Table II and XII-XXIV Sites are numbered using the sequence numbers from (Westin, E. H., et al., 1990, *Oncogene*, 5, 1117-1124) (GenBank Accession No. X52125); the sequence is derived from a longer c-*myb* cDNA isolate and thus is more representative of the full-length RNA.

Example 2: Selection of Ribozyme Cleavage Sites in Murine and Human *c-myb* mRNA.

To test whether the sites predicted by the computer-based RNA folding algorithm corresponded to accessible sites in c-*myb* RNA, 41 hammerhead sites were selected for analysis. Ribozyme target sites were chosen by comparing cDNA sequences of mouse and human c-*myb* (GenBank Accession No. X02774 and GenBank Accession No. X52125, respectively) and prioritizing the sites on the basis of overall nucleotide sequence homology. Hammerhead ribozymes were designed that could bind each target (see Figure 2C) and were individually analyzed by computer folding (Jaeger, J. A., et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86, 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from

consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Example 3: Screening Ribozyme Cleavage Sites by RNaseH Protection

Murine and human mRNA was screened for accessible cleavage sites by the method described generally in Draper et al., International PCT publication WO 93/23569, hereby incorporated by reference herein. Briefly, DNA oligonucleotides representing 41 potential hammerhead ribozyme cleavage sites were synthesized. A polymerase chain reaction was used to generate a substrate for T7 RNA polymerase transcription from human or murine *c-myb* cDNA clones. Labeled RNA transcripts were synthesized *in vitro* from the two templates. The oligonucleotides and the labeled transcripts were annealed, RNAseH was added and the mixtures were incubated for the designated times at 37° C. Reactions were stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved was determined by autoradiographic quantitation using a phosphor imaging system. The results are shown in Figures 7 and 8. From these data, 20 hammerhead ribozyme sites were chosen as the most accessible (see Table III).

Example 4: Chemical Synthesis and Purification of Ribozymes for Efficient Cleavage of *c-myb* RNA

Ribozymes of the hammerhead or hairpin motif were designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described in Usman *et al.*, 1987 *J. Am. Chem. Soc.*, 109, 7845 and in Scaringe et al., 1990 *Nucleic Acids Res.*, 18, 5433 and made use of

common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G_5 and a U for A_{14} (numbering from Hertel et al., 1992 *Nucleic Acids Res.*, 20, 3252). Hairpin ribozymes were synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Ribozymes were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). All ribozymes were modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-flouro, 2'-*O*-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17, 34). Ribozymes were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; See Usman *et al.*, Synthesis, deprotection, analysis and purification of RNA and ribozymes, filed May, 18, 1994, U.S.S.N. 08/245,736 the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in Table III.

<u>Example 5: Ribozyme Cleavage of Long Substrate RNA Corresponding to c-*myb* mRNA Target</u>

Hammerhead-type ribozymes which were targeted to the murine c-*myb* mRNA were designed and synthesized to test the cleavage activity at the 20 most accessible sites in *in vitro* transcripts of both mouse and human c-*myb* RNAs. The target sequences and the nucleotide location within the c-*myb* mRNA are given in Table II. All hammerhead ribozymes were synthesized with binding arm (Stems I and III; see Figure 2C) lengths of seven nucleotides. Two hairpin ribozymes were synthesized to sites 1632 and 2231. The relative abilities of these ribozymes to cleave both

murine and human RNAs is summarized in Table II. Ribozymes (1 μM) were incubated with ^{32}P-labeled substrate RNA (prepared as described in Example 3, approximately 20 nM) for 60 minutes at 37°C using buffers described previously. Intact RNA and cleavage products were separated by electrophoresis through polyacrylamide gels. The percentage of cleavage was determined by Phosphor Imager® quantitation of bands representing the intact substrate and the cleavage products.

Five hammerhead ribozymes (directed against sites 549, 575, 1553, 1597, and 1635) and one hairpin ribozyme (directed against site 1632) were very active; they cleaved >70% of both murine and human c-*myb* RNA in 60 minutes. Nine of the hammerhead ribozymes (directed against sites 551, 634, 936, 1082, 1597, 1721, 1724, 1895, and 1943) were intermediate in activity, cleaving > 50% of both murine and human c-*myb* RNA in 60 minutes. All of the sites cleaved by these active ribozymes were predicted to be accessible to ribozyme cleavage in Table II. Six hammerhead ribozymes and one hairpin ribozyme showed low activity on at least one of the substrates. The observed differences in accessibility between the two species of c-*myb* RNA demonstrate the sensitivity of ribozyme action to RNA structure and suggest that even when homologous target sequences exist, ribozymes may be excluded from cleaving that RNA by structural constraints. This level of specificity minimizes non-specific toxicity of ribozymes within cells.

<u>Example 6: Ability of Hammerhead Ribozymes to Inhibit Smooth Muscle Cell Proliferation.</u>

The ribozymes that cleaved c-*myb* RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as follows. Aortas from adult Sprague-Dawley rats were dissected, connective tissue was removed under a dissecting microscope, and 1 mm^2 pieces of

the vessel were placed, intimal side up, in a Petri dish in Modified Eagle's Medium (MEM) with the following additives: 10% FBS, 2% tryptose phosphate broth, 1% penicillin/streptomycin and 2 mM L-Glutamine. The smooth muscle cells were allowed to migrate and grow to confluence over a 3-4 week period. These primary cells were frozen and subsequent passages were grown at 37° C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum (FBS), and the following additives: 2 mM L-Glutamine, 1% penicillin/streptomycin, 1 mM sodium pyruvate, non-essential amino acids (0.1 mM of each amino acid), and 20 mM Hepes pH 7.4. Cells passed four to six times were used in proliferation assays. For the cell proliferation assays, 24-well tissue culture plates were prepared by coating the wells with 0.2% gelatin and washing once with phosphate-buffered saline (PBS). RASMC were inoculated at $1x10^4$ cells per well in 1 ml of DMEM plus 10% FBS and additives and incubated for 24 hours. The cells were subconfluent when plated at this density. The cells were serum-starved by removing the medium, washing once with PBS, and incubating 48-72 hours in DMEM containing 0.5% FBS plus additives.

In several other systems, cationic lipids have been shown to enhance the bioavailability of oligonucleotides to cells in culture (Bennet, C. F., et al., 1992, *Mol. Pharmacology*, 41, 1023-1033). In many of the following experiments, ribozymes were complexed with cationic lipids. The cationic lipid, Lipofectamine (a 3:1 (w/w) formulation of DOSPA (2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate) and dioleoyl phosphatidylethanolamine (DOPE)), was purchased from Life Technologies, Inc. DMRIE (*N*-[1-(2,3-ditetradecyloxy)propyl]-*N,N*-dimethyl-*N*-hydroxyethyl-ammonium bromide) was obtained from VICAL. DMRIE was resuspended in $CHCl_3$ and mixed at a 1:1 molar ratio with dioleoyl phosphatidylethanolamine (DOPE). The $CHCl_3$ was evaporated, the lipid was resuspended in water, vortexed

for 1 minute and bath sonicated for 5 minutes. Ribozyme and cationic lipid mixtures were prepared in serum-free DMEM immediately prior to addition to the cells. DMEM plus additives was warmed to room temperature (about 20-25°C), cationic lipid was added to the final desired concentration and the solution was vortexed briefly. RNA oligonucleotides were added to the final desired concentration and the solution was again vortexed briefly and incubated for 10 minutes at room temperature. In dose response experiments, the RNA/lipid complex was serially diluted into DMEM following the 10 minute incubation.

Serum-starved smooth muscle cells were washed twice with PBS, and the RNA/lipid complex was added. The plates were incubated for 4 hours at 37°C. The medium was then removed and DMEM containing 10% FBS, additives and 10 μM bromodeoxyuridine (BrdU) was added. In some wells, FBS was omitted to determine the baseline of unstimulated proliferation. The plates were incubated at 37°C for 20-24 hours, fixed with 0.3% H_2O_2 in 100% methanol, and stained for BrdU incorporation by standard methods. In this procedure, cells that have proliferated and incorporated BrdU stain brown; non-proliferating cells are counter-stained a light purple. Both BrdU positive and BrdU negative cells were counted under the microscope. 300-600 total cells per well were counted. In the following experiments, the percentage of the total cells that have incorporated BrdU (% cell proliferation) is presented. Errors represent the range of duplicate wells. Percent inhibition then is calculated from the % cell proliferation values as follows: % inhibition = 100 - 100((Ribozyme - 0% serum)/(Control - 0% serum)).

Six hammerhead ribozymes, including the best five ribozymes from the *in vitro* RNA cleavage test (directed against sites 549, 575, 1553, 1598, and 1635) and one with intermediate cleavage levels (directed against site 1597) and their catalytically inactive controls were synthesized and purified as described above. The ribozymes were

delivered at a concentration of 0.3 μM, complexed with DMRIE/DOPE such that the cationic lipid charges and the anionic RNA charges were at 1:1 molar ratio. The results, shown in Table IV, demonstrate a considerable range in the efficacy of ribozymes directed against different sites. Five of the six hammerhead ribozymes (directed against sites 549, 575, 1553, 1597, and 1598) significantly inhibit smooth muscle cell proliferation. The control, inactive ribozymes that cannot cleave c-*myb* RNA due to alterations in their catalytic core sequence fail to inhibit rat smooth muscle cell proliferation. Thus, inhibition of cell proliferation by these five hammerhead sequences is due to their ability to cleave c-*myb* RNA, and not because of any antisense activity. The sixth ribozyme (directed against site 1635) fails to function in smooth muscle cells. This ribozyme cleaved c-*myb* RNA very efficiently *in vitro*. In this experiment, 10% FBS (no ribozyme added) induced 64 ± 1% proliferation; 0% FBS produced a background of 9 ± 1% proliferation.

<u>Example 7: Ability of exogenously delivered hairpin ribozyme against c-myb to inhibit vascular smooth muscle cell proliferation</u>

In addition to the hammerhead ribozymes tested above, a bipartite hairpin ribozyme (Chowrira, B. M., supra, 1992, <u>*Nucleic Acids Res.*</u>, 20, 2835-2840) was identified that also cleaves c-*myb* RNA. The effect of this ribozyme on smooth muscle cell proliferation was tested. Ribozymes were delivered at the indicated doses with Lipofectamine at a 1:1 charge ratio. In this experiment, 10% FBS (no ribozyme) induced 87 ± 1% proliferation; 0% FBS produced 5 ± 1% proliferation. The results of a dose-response experiment are shown in Table V. In this example, the control was an irrelevant hammerhead ribozyme. The irrelevant ribozyme control contains the same catalytic core sequences, but has binding arms that are directed to a cellular RNA that is not required for smooth muscle cell

proliferation. This control failed to significantly inhibit cell proliferation, demonstrating the sequence specificity of these ribozymes. Another control that could be run is an irrelevant catalytically active ribozyme having the same G:C content as the test ribozyme.

Example 8: Ribozymes inhibit proliferation of rat smooth muscle cells in a dose-dependent fashion.

If the inhibition of proliferation observed in Example 6 is caused by the ribozymes, the level of inhibition should be proportional to the dose of RNA added. Rat aortic smooth muscle cells were assayed for proliferation in the presence of differing doses of two hammerhead ribozymes. The results shown in Table VI indicate that two hammerhead ribozymes that cleave *c-myb* RNA at sites 575 and 549 inhibit SMC proliferation in a dose-dependent fashion. Ribozymes were delivered with the cationic lipid, Lipofectamine at a 1:1 charge ratio. In this experiment, 10% FBS (no ribozyme) gave 92 ± 1% proliferation; 0% FBS gave 6 ± 1% proliferation. The control is an active ribozyme directed against an irrelevant mRNA target and shows no inhibition over the dose range tested. The control ribozyme contains the same catalytic core sequences as the active ribozymes but differs in its binding arm sequences (stems I and III in Figure 2c). Thus, ribozyme inhibition of smooth muscle cell proliferation requires sequence-specific binding by the hammerhead arms to *c-myb* mRNA.

Example 9: Delivery of a *c-myb* Ribozyme With Different Cationic Lipids

The experiment in Table VII shows the response of rat smooth muscle cells to a hammerhead ribozyme that cleaves *c-myb* RNA at site 575 delivered with two different cationic lipids, DMRIE and Lipofectamine. Similar efficacy is observed with either lipid. 10% FBS (no

ribozyme) induced 78 ± 2% proliferation; 0% FBS produced a background of 6 ± 1% proliferation.

Example 10: Effect of varying arm-lengths on ribozyme activity.

The exact configuration of each ribozyme can be optimized by altering the length of the binding arms (stems I and III, see Figure 2C). The length of the binding arms may have an effect on both the binding and the catalytic cleavage step (Herschlag, D., 1991, *Proc. Natl. Acad. Sci. U S A*, 88, 6921-5). For example, Table VIII shows the ability of arm length variants of c-myb hammerhead 575 to inhibit SMC proliferation. Note that the dose used in this experiment (0.1 μM) is 3-fold lower than in previous experiments. At this concentration, the 7/7 arm variant gives relatively little inhibition. In this case, the degree of inhibition increases with concomitant increases in arm length.

The optimum arm length may be site-specific and should be determined empirically for each ribozyme. Towards this end, hammerhead ribozymes target with 7 nucleotide binding arms (7/7) and ribozymes with 12 nucleotide binding arms (12/12) targeted to three different cleavage sites were compared.

Ribozymes were delivered at 0.2 μM with the cationic lipid DMRIE at a 1:1 charge ratio of oligonucleotide to cationic lipid as described in Example 6. The data are shown below in Table IX. As can be seen, all three ribozymes demonstrated enhanced inhibition of smooth muscle cell proliferation with twelve nucleotide binding arms. Each ribozyme showed greater inhibition than its catalytically inactive control, again demonstrating that the ribozymes function via their ability to cleave c-*myb* RNA. In this experiment, 10% stimulation resulted in 54 ± 2 % cell proliferation; unstimulated cells showed 8 ± 0.5 % cell proliferation.

Example 11: Effect of chloroquine on ribozyme activity.

A number of substances that effect the trafficking of macromolecules through the endosome have been shown to enhance the efficacy of DNA delivery to cells. These include, but are not limited to, ammonium chloride, carbonyl cyanide *p*-trifluoromethoxy phenyl hydrazone (FCCP), chloroquine, monensin, colchicine, and viral particles (Cotten, M. et al.,1990, *Proc. Natl. Acad. Sci. USA* , 87, 4033-4037; Cotten, M. et al.,1993, *J. Virol.* , 67, 3777-3785; Cotten, M. et al.,1992, *Proc. Natl. Acad. Sci. USA* , 89, 6094-6098; Cristiano, R. J. et al.,1993, *Proc. Natl. Acad. Sci. U S A* , 90, 2122-6; Curiel, D. T. et al.,1991, *Proc. Nat. Acad. Sci. USA* , 88, 8850-8854; Ege, T. et al.,1984, *Exp. Cell Res.* , 155, 9-16; Harris, C. E. et al.,1993, *Am. J. Respir. Cell Mol. Biol.* , 9, 441-7; Seth, P. et al.,1994, *J. Virol.* , 68, 933-40; Zenke, M. et al.,1990, *Proc. Natl. Acad. Sci. USA* , 87, 3655-3659). It is thought that DNA is taken up by cells by endocytosis, resulting in DNA accumulation in endosomes (Akhtar, S. and Juliano, R. L.,1992, *Trends Cell Biol.* , 2, 139-144). Thus, the above agents may enhance DNA expression by promoting DNA release from endosomes. To determine whether such agents may augment the functional delivery of RNA and ribozymes to smooth muscle cells, the effects of chloroquine on ribozyme inhibition of smooth muscle cell proliferation were assessed. A ribozyme with twelve nucleotide binding arms that cleaves c-*mby* RNA was delivered to rat smooth muscle cells as described in Example 6 (0.2 μM ribozyme complexed with DMRIE/DOPE at a 1:1 charge ratio). In some cases, 10 μM chloroquine was added upon stimulation of the cells. The addition of chloroquine had no effect on untreated cells (stimulation with 10% serum in the presence or absence of chloroquine resulted in 80.5 $\pm$ 1.5 % and 83 $\pm$ 2% cell proliferation, respectively; unstimulated cells with and without chloroquine showed 7 $\pm$ 0.5% and 7 $\pm$ 1% cell proliferation, respectively). As shown in Table X below, addition of

chloroquine augments ribozyme inhibition of smooth muscle cell proliferation two- to three-fold.

Example 12: Effect of a hammerhead ribozyme on human smooth muscle cell proliferation.

The hammerhead ribozyme that cleaves human c-*myb* RNA at site 549 was tested for its ability to inhibit human aortic smooth muscle cell proliferation. The binding site for this ribozyme is completely conserved between the mouse and human cDNA sequences. Human aortic smooth muscle cells (AOSMC) were obtained from Clonetics and were grown in SmGM (Clonetics®). Cells from passage five or six were used for assays. Conditions for the proliferation assay were the same as for the rat cells (see Example 6), except that the cells were plated in SmGM and starved in SmBM plus 0.5% FBS. The ribozyme that cleaves site 549 was delivered at varying doses complexed with the cationic lipid DMRIE at a 1:1 charge ratio. In this experiment, 10% FBS (no ribozyme) induced 57 ± 7% proliferation; the uninduced background was 6 ± 1% proliferation. The results in Table XI show that inhibition is observed over a similar concentration range as was seen with rat smooth muscle cells.

Example 13: Inhibition by direct addition of a modified, stabilized ribozyme.

A hammerhead ribozyme that cleaves site 575 was chemically synthesized with 12 nucleotide binding arms (sequence ID NO. 127, in Table III). Chemically modified nucleotides were incorporated into this ribozyme that have been shown to enhance ribozyme stability in serum without greatly impacting catalytic activity. (See Eckstein *et al.*, *International Publication* No. WO 92/07065, Perrault *et al.*, 1990, Nature , 344, 565-568, Pieken,W. *et al.* 1991, *Science* , 253, 314-317, Usman,N.; Cedergren,R.J., 1992, *Trends in Biochem. Sci.*, 17, 334-339, Usman,N. *et al.* *US Patent Application 07/829,729*, and Sproat,B.

European Patent Application 92110298.4 describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.) The modifications used were as follows. All the nucleotides of the ribozyme contained 2´-*O*-methyl groups with the following exceptions: U_4 and U_7 contained 2´-amino substitutions; G_5, A_6, G_8, G_{12}, and $A_{15.1}$ were 2´-OH ribonucleotides (numbering as in Figure 1). An inactive ribozyme was chemically synthesized in which G_5 and A_{14} were substituted with 2´-*O*-methyl U. Ribozymes were added to rat smooth muscle cells at the indicated concentrations as per Example 6 except that cationic lipids were omitted. Proliferation was assessed by BrdU incorporation and staining. Table XII shows that the modified ribozyme is capable of inhibiting rat smooth muscle cell proliferation without addition of cationic lipids. In this experiment, 10% serum induced 45 ± 2 % proliferation while uninduced cells showed a background of 2.3 ± 0.1 % proliferation.

<u>Optimizing Ribozyme Activity</u>

As demonstrated in the above examples, ribozymes that cleave c-*myb* RNA are capable of inhibiting 50% of the smooth muscle cells from proliferating in response to serum. This level of inhibition does not represent the maximal effect obtainable with the ribozymes; in each dose response experiment, the highest dose produced the greatest extent of inhibition. Thus, optimizing activity of the ribozyme within the cells and/or optimizing the delivery of the ribozyme to the cells is expected to increase the extent of inhibition.

Tables VIII and IX demonstrate one means of optimizing ribozyme activity. By altering the length of the ribozyme binding arms (stems I and III, see Figure 2c), the ability of the ribozyme to inhibit smooth muscle cell proliferation is greatly enhanced. Ribozymes with increasing arm lengths will be synthesized either chemic-

ally in one or two parts (see above and see Mamone, U.S. Serial No. 07/882,689, filed May 11, 1992, hereby incorporated by reference herein) or by *in vitro* transcription (see Cech et al., U.S. Patent 4,987,071). Ribozymes are chemically synthesized with modifications that prevent their degradation by serum ribonucleases (as described in Example 13, above). When synthesized in two parts, the fragments are ligated or otherwise juxtaposed as described (see original application and Mamone, supra). The effects of the ribozymes on smooth muscle cell proliferation are assessed as in Examples 6 and 12, above. As the length of stems I and III can affect both hybridization to the target and the catalytic rate, the arm length of each ribozyme will be optimized for maximal inhibitory effect in cells. Similarly, the precise sequence of modified nucleotides in the stabilized ribozyme will affect the activity in cells. The nature of the stabilizing modifications will be optimized for maximal inhibitory effect in cells. In each case, activity of the ribozyme that cleaves c-*myb* RNA will be compared to the activity of its catalytically inactive control (substitution of 2′-*O*-methyl U for G_5 and a 2′-*O*- methyl U for A_{14}) and to a ribozyme targeted to an irrelevant RNA (same catalytic core, with appropriate modifications, but different binding arm sequences).

Sullivan, *et al.*, supra, describes the general methods for delivery of enzymatic RNA molecules. The data presented in Example 9 indicate that different cationic lipids can deliver active ribozymes to rat smooth muscle cells. In this example, 0.6 μM ribozyme delivered with Lipofectamine produced the same inhibitory effect as 0.3 μM ribozyme delivered with DMRIE. Thus, DMRIE is twice as efficacious as Lipofectamine at delivering active ribozymes to smooth muscle cells. There are a number of other cationic lipids known to those skilled in the art that can be used to deliver nucleic acid to cells, including but not limited to dioctadecylamidoglycylspermine (DOGS),

dioleoxltrimetylammonium propane (DOTAP), N-[1-(2,3-dioleoyloxy)-propyl]-n,n,n-trimethylammoniumchloride (DOTMA), N-[1-(2,3-dioleoyloxy)-propyl]-N,N-dimethyl-N-hydroxyethylammonium bromide (DORIE), and N-[1-(2,3-dioleoyloxy)propyl]-N,N-dimethyl-N-hydroxypropylammonium bromide (DORIE-HP). Experiments similar to those performed in Example 9 are used to determine which lipids give optimal delivery of ribozymes to smooth muscle cells. Other such delivery methods are known in the art and can be utilized in this invention.

The data described in Example 11 show that ribozyme delivery and efficacy may be augmented by agents that disrupt or alter cellular endosome metabolism. Chloroquine was shown to increase the ability of a ribozyme to inhibit smooth muscle cell proliferation by 2- to 3-fold. Experiments similar to those described in Example 11 can be performed to determine the optimal concentration of chloroquine to be used to augment delivery of ribozymes alone (as in Example 13), or delivery in the presence different cationic lipids (as in Example 9 and described above) or with other delivery agents (as described below). Other agents that disrupt or alter endosomes known to those familiar with the art can be used to similarly augment ribozyme effects. These agents may include, but are not limited to, ammonium chloride, carbonyl cyanide *p*-trifluoromethoxy phenyl hydrazone (FCCP), chloroquine, monensin, colchicine, amphipathic peptides, viral proteins, and viral particles. Such compounds may be used in conjunction with ribozymes as described above, may be chemically conjugated directly to ribozymes may be chemically conjugated to liposomes, or may be incorporated with ribozymes in liposome particles (see Sullivan, *et al.*, supra, incorporated by reference herein).

The data presented in Example 13 indicate that the proliferation of smooth muscle cells can be inhibited by the direct addition of chemically stabilized ribozymes.

Presumably, uptake is mediated by passive diffusion of the anionic nucleic acid across the cell membrane. In this case, efficacy could be greatly enhanced by directly coupling a ligand to the ribozyme. The ribozymes are then delivered to the cells by receptor-mediated uptake. Using such conjugated adducts, cellular uptake can be increased by several orders of magnitude without having to alter the phosphodiester linkages necessary for ribozyme cleavage activity.

Alternatively, ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. The RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. Alternative routes of delivery include, but are not limited to, intramuscular injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan, *et al.*, <u>supra</u> and Draper, *et al.*, <u>supra</u> which have been incorporated by reference herein.

<u>Example 14: Phosphorothioate linkages enhance the ability of ribozymes to inhibit smooth muscle cell proliferation.</u>

As the applicant had shown in Example 13, the hammerhead (HH) ribozyme that cleaves *c-myb* RNA at site 575 can be modified to confer resistance to nucleases while maintaining catalytic activity (see also Usman et al., <u>supra</u>). To identify ribozymes with optimal activity in cells, several different chemically-modified ribozymes were directly compared for inhibition of rat smooth muscle cell proliferation. Non-limiting examples of chemically-modified ribozymes used are diagrammed in Figure 9A. One

ribozyme (designated "2'-*O*-methyl") contains ribonucleotide residues at all positions except the 5 terminal nucleotides of each target binding arm (Stems I and III). The ribozyme designated "2'-*O*-methyl P=S" in addition contains five phosphorothioate linkages between the terminal nucleotides in each target binding arm. The ribozyme termed "2'-*C*-allyl iT" contains thirty 2'-*O*-methyl nucleotides as specified in Example 13. The ribozyme also contains 2'-*C*-allyl U (Usman et al., 1994 *Nucleic Acids Symp. Ser.* 31, 163) at the U4 position and 2'-*O*-methyl U at the U7 position and a 3'-3'-linked inverted thymidine (Ortigao et al., 1992 *Antisense Res. & Development* 2, 129; Seliger et al., Canadian Patent Application No. 2,106,819) at the 3' end of the molecule (referred to as 2'-*C*-allyl iT). The fourth ribozyme contains the same 2'-*O*-methyl and 2'-*C*-allyl residues described above with the addition of 5 phosphorothioate linkages between the terminal nucleotides in each target binding arm (referred to as "2'-*C*-allyl P=S").

Ribozymes were delivered to smooth muscle cells as cationic lipid complexes (Sullivan et al., *supra*). In this example, the cationic lipid, Lipofectamine (GIBCO-BRL), was used at a charged lipid concentration of 3.6 μM (see Examples 6 and 9). Active versus inactive forms of each ribozyme were compared to determined whether inhibition is mediated specifically by ribozyme cleavage. As shown in Figure 9B, the ribozyme synthesized with the 2'-*C*-allyl modification and the phosphorothioate linkages demonstrated enhanced inhibition of smooth muscle cell proliferation. The catalytically inactive form of the ribozyme had little effect on cell proliferation; thus, the inhibition observed requires the catalytic activity of the ribozyme. In contrast, ribozymes without the stable 2'-*O*-methyl- and 2'-*C*-allyl-modified catalytic core (2'-*O*-methyl and 2'-*O*-methyl P=S) at best showed only modest inhibition of smooth muscle cell proliferation. The stable core chemistry alone was not sufficient to greatly

enhance ribozyme-mediated inhibition; without terminal P=S linkages, the 2´-*C*-allyl-modified ribozyme showed very little specific inhibition when compared to its inactive ribozyme control. These results demonstrate that certain chemical modifications greatly enhance the ability of exogenously-delivered ribozymes to cleave *c-myb* RNA and impact cell proliferation.

Example 15: Dose response of the chemically modified ribozyme.

Varying doses of the 2´-*C*-allyl P=S-modified ribozyme were delivered to rat aortic smooth muscle cells as described above. As in previous examples, percent inhibition was calculated by comparing the effects of the active ribozyme to the effects of the inactive ribozyme. As shown in Figure 10, the ribozyme concentration at which cell proliferation is inhibited by 50% (IC_{50}) is approximately 70 nM. From day to day, the IC_{50} varies between 25 and 100 nM.

Example 16: Direct comparison of the effects of ribozymes and antisense DNA.

Ribozymes are thought to be more specific reagents for the inhibition of gene expression than antisense oligonucleotides due to their catalytic activity and strict sequence requirements around the site of cleavage (Castanotto et al., 1994 *Adv. in Pharmacol.* 25, 289) . To test this hypothesis, ribozyme activity was directly compared to the activity of phosphorothioate DNA oligonucleotides that target the same site in the *c-myb* mRNA. The ribozyme used was the 2´-*C*-allyl P=S-modified ribozyme described in Example 14, above. This ribozyme binds to a 15 nucleotide long region of the *c-myb* mRNA. Thus, a 15 nucleotide antisense phosphorothioate DNA molecule was prepared. A phosphorothioate DNA oligonucleotide with a randomly scrambled sequence of the same 15 nucleotides and a 2´-*C*-allyl P=S-modified ribozyme with randomly scrambled

target binding arm sequences were synthesized as controls (by comparison to the murine c-*myb* cDNA sequence, the scrambled controls would not be expected to bind any region of the c-*myb* mRNA). Since longer phosphorothioate DNA oligonucleotides are often utilized as antisense inhibitors (for a review see Wagner, 1994 *Science* 372, 333), a symmetrically placed, 25 nucleotide phosphorothioate DNA antisense oligonucleotide and its scrambled sequence control were also synthesized. The ribozymes and the antisense oligonucleotides were delivered to rat smooth muscle cells as complexes with the cationic lipid, Lipofectamine, and serum-stimulated smooth muscle cell proliferation was measured subsequently.

As shown in Figure 11, the 2´-*C*-allyl P=S-modified ribozyme demonstrated greater inhibition of smooth muscle cell proliferation than either of the antisense oligonucleotides. Furthermore, the scrambled arm ribozyme and inactive ribozyme controls demonstrated less non-specific inhibition than either of the scrambled sequence antisense control oligonucleotides. In fact, the non-specific inhibition demonstrated by the 25 nucleotide phosphorothioate molecule completely masked any specific effect of the antisense molecule. Similar results have been obtained with phosphorothioate DNA targeting other sites in the c-*myb* mRNA. Thus, a ribozyme that cleaves c-*myb* RNA is a more potent and more specific inhibitor of smooth muscle cell proliferation than phosphorothioate antisense DNA molecules.

<u>Example 17: Chemically-modified ribozymes targeting different sites in the c-*myb* mRNA specifically inhibit smooth muscle cell proliferation.</u>

If the observed inhibition of smooth muscle cell proliferation is mediated by ribozyme cleavage of c-*myb* mRNA, then other ribozymes that target the same mRNA should have the same effect. Two other ribozymes targeting two disparate sites in the c-*myb* mRNA (sites 549 and

1553, ribozyme Seq. ID Nos. 102 and 112) were synthesized with the 2´-*C*-allyl P=S modifications as described in Example 14. Inactive ribozyme controls also were synthesized corresponding to each new target sequence. Chemically-modified ribozymes targeting sites 549, 575, and 1553 were delivered to rat smooth muscle cells and their ability to inhibit serum-stimulated cell proliferation was assessed. Equivalent levels of inhibition are obtained with active ribozymes targeting sites 549, 575 and 1553 (see Figure 12). None of the inactive ribozymes inhibited cell proliferation. Active ribozymes targeting other mRNA sequences not present in c-*myb* or ribozymes with scrambled arm sequences also fail to inhibit smooth muscle cell proliferation (see Figure 12). Thus, inhibition of cell proliferation requires a catalytically active ribozyme that can bind to accessible c-*myb* mRNA sequences and is likely due to the reduction of c-*myb* mRNA levels by ribozyme cleavage.

Examples 18 and 19 describe experiments designed to determine the position and minimum number of phosphorothioate residues required for efficacy.

Example 18: Effect of position of phosphorothioate linkages on ribozyme inhibition.

Ribozymes targeting c-*myb* site 575 were synthesized with the 2´-*C*-allyl modification and with phosphorothioate linkages between various nucleotides in the ribozyme. One ribozyme contained a total of 10 phosphorothioate linkages, 5 in Stem I and 5 in Stem III, identical to the ribozyme described in Examples 14 through 17 above (referred to as 10 P=S 5´ and 3´ in Figure 13A). One ribozyme contained only 5 phosphorothioate linkages in Stem III (5 P=S 3´ in Figure 13A). Another ribozyme contained 5 phosphorothioate linkages between the 6 nucleotides comprising the last base pair of stem II and the GAAA loop (5 P=S loop in Figure 13A). The fourth ribozyme contained 5 phosphorothioate linkages in stem I

(5 P=S 5′ in Figure 13A). The latter two ribozymes also were synthesized with the 3′-3′ thymidine at the 3′ end to help protect the ribozyme from 3′ exonucleases (Ortigao et al., 1992 *Antisense Res. & Development* 2, 129; Seliger et al., Canadian Patent Application No. 2,106,819). The structure of these four different ribozymes is diagrammed in Figure 13A. Inactive ribozyme controls were synthesized for each individual ribozyme. The active and inactive ribozymes were applied to rat smooth muscle cells as RNA/Lipofectamine complexes and their effects on cell proliferation were measured.

Referring to Figure 13B, the ribozyme containing 5 phosphorothioate linkages in Stem I and the 3′ inverted thymidine inhibited smooth muscle cell proliferation as well as the parent ribozyme with 10 total phosphorothioate linkages. None of the other ribozymes demonstrated significant differences between active and inactive controls. Therefore, the 3′ inverted T can effectively substitute for the 5 phosphorothioate linkages in Stem III. Phosphorothioate linkages in the loop position lead to non-specific inhibition of smooth muscle cell proliferation, while phosphorothioate linkages in Stem I are necessary for enhanced efficacy in cells. Additionally, these results suggest that 3′-end modifications, such as iT, is desirable to minimize the amount of phosphorothioate contained in the ribozymes in order to minimize toxicity and facilitate chemical synthesis, while maintaining protection from endogenous 3′-exonuclease digestion.

Example 19: Minimizing phosphorothioate linkages in Stem I.

Fewer phosphorothioate linkages in the ribozyme will reduce the complexity and cost of chemical synthesis. Furthermore, phosphorothioate DNA molecules are known to have some undesirable and non-specific effects on cellular functions (for a review see Wagner, *supra*); reducing the

phosphorothioate linkages in these RNA molecules is expected to enhance their specificity. A series of ribozymes targeting c-*myb* were synthesized to determine how many phosphorothioate linkages in Stem I are required for optimal ribozyme activity. The ribozymes contained 5, 4, 3, 2, or 1 phosphorothioate linkage(s) in Stem I, beginning with the phosphodiester bond between the first and second nucleotides and proceeding 3´. Each ribozyme contained the 2´-*O*-methyl modifications, the U_4 2´-*C*-allyl nucleotide, and the inverted T nucleotide at the 3´ end as described above. Activity of each of these ribozymes was compared to the activity of the ribozyme with 10 phosphorothioate linkages, 5 each in Stems I and III (referred to as 10 P=S in Figure 14). Active and inactive ribozymes were applied to rat smooth muscle cells as complexes with Lipofectamine and their effects on smooth muscle cell proliferation were measured in two separate experiments. The results are diagrammed in Figure 14. Ribozymes with 10, 5, and 4 phosphorothioate linkages showed equivalent efficacy. Ribozymes with fewer than four phosphorothioate linkages also showed efficacy, but the level of inhibition of smooth muscle cell proliferation was modestly reduced.

<u>Example 20: Varying the length of Stems I and III</u>

Ribozymes that cleave c-*myb* RNA at position 575 were synthesized with varying arm lengths. Each ribozyme contained 4 phosphorothioate linkages at the 5´ end, 2´-*O*-methyl and 2´-*C*-allyl modifications and an inverted thymidine nucleotide at the 3´ end as described above. Figure 15 shows the effects of these ribozymes upon rat smooth muscle cell proliferation. Ribozymes were delivered at 100 nM with cationic lipid. Ribozymes with 6/6, 7/7 and 5/10 arms (where x/y denotes the nucleotides in Stem I/nucleotides in Stem III; see Figure 2) all showed comparable efficacy. As shown in Figure 15, ribozymes with longer arm lengths tended to demonstrate more non-

specific inhibition (the inactive ribozyme controls with longer binding arms inhibited smooth muscle cell proliferation) when compared to ribozymes with shorter binding arms. From these data, it appears that ribozymes with 6/6, 7/7, 5/10, 10/5, 8/8 and 10/10 nucleotide arms all specifically inhibit smooth muscle cell proliferation, optimal inhibition, however, is observed with 6/6, 7/7 and 5/10 nucleotide arms.

Example 21: Ribozymes with different modified nucleotides inhibit smooth muscle cell proliferation.

Ribozymes containing seven nucleotides in both Stems I and III, four phosphorothioate residues at the 5´ end and a 3´-3´ inverted thymidine at the 3´ end, were synthesized with various modified nucleotides at the U_4 and U_7 positions within the core of a HH ribozyme. All of the modified catalytic core chemistries retained ribozyme activity and demonstrated enhanced stability to serum nucleases (Usman et al., 1994 *supra*). The ribozyme termed U4 2´-*C*-allyl contains a 2´-*C*-allyl uridine at the U_4 position and a 2´-*O*-methyl nucleotide at the U_7 position. The ribozyme termed U4,U7 2´-amino contains a 2´-amino nucleotide at both U4 and U7. The ribozyme termed U4 2´-fluoro contains a 2´-fluoro-modified nucleotide at U4 and 2´-*O*-methyl at U7. The ribozyme termed U4 6-methyl contains a 6-methyl uridine nucleotide at U4 and 2´-*O*-methyl at U7. The ribozyme termed U4 deoxyabasic contains a deoxyribose moeity and lacks a base at U4 (Beigelman et al., 1994 *Bioorganic & Med. Chem. Letters* 4, 1715) and 2´-*O*-methyl at U7. Active and inactive versions of each of the chemically-modified ribozymes were applied to rat smooth muscle cells using Lipofectamine as described above. As diagrammed in Figure 16, all of the nuclease-stable, chemically-modified ribozymes demonstrated significant inhibition of rat smooth muscle cell proliferation. Thus, the requirements for ribozyme activity in smooth muscle cells appear to be a catalytically core that is

modified to minimize endonucleolytic degradation and modifications at the 5´ and 3´ ends which may prevent exonucleolytic degradation.

Chemical modifications described in this invention are meant to be non-limiting examples, and those skilled in the art will recognize that other modifications (base, sugar and phosphate modifications) to enhance nuclease stability of a ribozyme can be readily generated using standard techniques and are hence within the scope of this invention.

Example 22: Ribozyme inhibition of pig smooth muscle cell proliferation.

Of the commonly used animal models of intimal hyperplasia after balloon angioplasty, the pig model is believed to be most predictive of human disease (Steele et al., 1985 *Circ. Res.* 57, 105; Ohno et al., 1994 *Science* 265, 781; Baringa, 1994 *Science* 265, 738). Therefore, we wished to assess the ability of c-*myb* ribozymes to inhibit pig smooth muscle cell proliferation. Yucatan pig smooth muscle cells (YSM) were obtained from Dr. Elizabeth Nabel (University of Michigan Medical Center) and were grown in Dulbecco's modified Eagle's medium as described (see Example 6). The YSM cells were starved for 72 hours in DMEM with 0.1% FBS. Active and inactive ribozymes (four phosphorothioate linkages at the 5´ end, 2´-*C*-allyl-modified core and 3´-3´ inverted thymidine at the 3´ end) were applied as RNA/Lipofectamine® complexes as described in the above examples. Proliferation was stimulated with serum and assessed by BrdU incorporation. Figure 17 shows that a ribozyme dose of as low as 75 nM can inhibit pig smooth muscle cell proliferation by as much as 60%. The same chemical modifications of the ribozymes (2´-modified, stable core, 5´ phosphorothioate linkages and 3´ inverted thymidine) are required to obtain significant and reproducible inhibition of pig smooth muscle cell proliferation

as were shown to be required for inhibition of rat cells in the above Examples.

Example 23: Ribozyme inhibition of human smooth muscle cell proliferation.

In Example 12, we demonstrated that a minimally modified ribozyme directed against *c-myb* site 549 could significantly inhibit human smooth muscle cell proliferation. The 2´-*C*-allyl and phosphorothioate-modified ribozyme targeting *c-myb* site 575 characterized above was applied to human smooth muscle cells as RNA/Lipofectamine® complexes. Inactive ribozyme and inactive, scrambled arm ribozymes were applied as controls. At 200 nM, the active ribozyme inhibits human smooth muscle proliferation by greater than 75% while the inactive ribozyme inhibits proliferation by only 38%. The ribozyme with scrambled binding arm sequences fails to inhibit. At 100 nM, the active ribozyme still demonstrates significant inhibition while neither the inactive or scramble controls inhibit cell proliferation (see Figure 18). Thus, the active ribozyme identified in these studies mediates significant inhibition of human smooth muscle cell proliferation and represents a novel therapeutic for restenosis and/or vascular disease.

Example 24: Delivery of *c-myb* ribozymes to vessels *in vivo*.

The ribozyme that cleaves *c-myb* RNA at site 575 was synthesized in two parts (Mamone, *supra*), the internal 5´ end was labeled with ^{33}P using polynucleotide kinase and the two fragments were ligated with RNA ligase. The resulting RNA was an intact ribozyme with an internal ^{33}P label. This internally-labeled ribozyme was delivered to balloon injured rat carotid arteries as described (Simons et al., 1992 *Nature* 359, 67). Rats were anesthetized and the carotid artery was surgically exposed. The external carotid was dissected and a 2F Fogarty balloon catheter

was inserted and directed into the carotid artery. Injury was caused by repeated (3 times) inflation and retraction of the balloon. The injured region was isolated by ligatures and a cannula was inserted in the external carotid. Ribozymes alone (two rat vessels) or ribozyme/Lipofectamine® complexes (two rat vessels) were applied to the injured vessel through the cannula and were left in the vessel for twenty minutes. After application, blood flow was restored by removal of the ligatures for five minutes and the vessels were harvested and processed as described below.

Half of the vessel was frozen in liquid nitrogen, crushed into a fine powder, and RNA was extracted using standard protocols. The extracted RNA was applied to a denaturing polyacrylamide gels and subjected to electrophoresis. Autoradiography of the gel permitted detection of the ^{33}P label; the amount of radioactivity in each band was quantitated using a Phosphor-imaging system. The amount of extracted and intact ribozyme was calculated by direct comparison to labeled ribozyme controls run on the same gel. The percentage of the ribozyme delivered intact could be estimated by quantifying the percentage of label that co-migrates with the intact ribozyme controls. After delivery of ribozymes in phosphate-buffered saline (PBS), 3% of the ^{33}P label was recovered from the rat vessels and >90% of the label was present in the form of intact ribozyme. After delivery of ribozyme in RNA/Lipofectamine complexes, 10 to 11% of the ^{33}P label was recovered from the rat vessels and 20 to 90% of the label was present in the form of intact ribozyme. The significant uptake of the intact ribozyme demonstrates that local delivery of modified ribozymes to arterial walls is feasible.

The other half of each vessel was fixed in PBS-buffered 2% glutaraldehyde, sectioned onto slides and coated with emulsion. After autoradiography for four days, the emulsion was developed and the sections were

stained with hematoxylin and eosin by standard techniques (Simons et al., 1992 _supra_). Inspection of the sections showed a majority of the grains present over the medial smooth muscle cells after application of the ribozyme. Some ^{33}P label could be detected in the underlying adventitia as well. Similar density and distribution of grains was observed when the ribozyme was delivered with or without Lipofectamine. These data demonstrate that ribozyme can penetrate the injured vessel wall and is in close apposition or within the underlying medial smooth muscle cells. Thus, therapeutic ribozymes can be locally delivered to vessels for the treatment of vascular disease.

Similar experiments were performed in pig iliofemoral vessels. After balloon injury, a ribozyme, internally labeled with ^{33}P as described above, was delivered with a double balloon catheter device (Nabel and Nabel, _supra_; Ohno et al., 1994 _supra_). After 20 minutes, blood flow was restored by deflating the balloons. The vessels were harvested after an additional hour or the surgical injuries were sutured and the vessels harvested one day later. Harvested vessels were sectioned, subjected to autoradiography and stained. One hour after delivery, the majority of the ^{33}P label could be detected in the media, overlying or within smooth muscle cells. Some label was also detected at the luminal surface of the vessel and in the adventitial tissue. One day after delivery, grains could be still be detected associated with remaining medial smooth muscle cells. No major differences in density or distribution was observed between ribozymes delivered with or without Lipofectamine®. These data demonstrate that ribozymes can be locally delivered to smooth muscle cells of injured vessels in a large animal model that is clinically relevant to human vascular disease.

Example 25: Ribozyme-mediated decrease in the level of *c-myb* RNA in rat smooth muscle cells.

To determine whether a ribozyme catalyzes the cleavage of *c-myb* RNA in a mammalian cell, applicant has used a sensitive quantitative competitive polymerase chain reaction (QCPCR) to assay the level of *c-myb* RNA in rat smooth muscle cells treated with either catalytically active or inactive ribozyme.

Rat smooth muscle cells (RASMC) were treated with ribozymes as described above. Following the ribozyme treatment for 4h, cells were stimulated with 10% serum (in the presence or absence of BrdU). After 24h, cells were harvested for further analysis. Cells, that were treated with BrdU, were assayed for proliferation as described above. Cells, that were not treated with BrdU, were used for the QCPCR assay.

The following is a brief description of the QCPCR technique used to quantitate levels of c-myb mRNA from RASMC, normalizing to the housekeeping gene, GAPDH. This method was adapted from Thompson et al, Blood 79:1692, 1992. Briefly, total RNA was isolated from RASMC using the Guanidinium isothiocyanate technique of Chomczynski and Sacchi (Analytical Biochemistry, 162:156, 1987). In order to construct a deletion competitor and control wild-type RNA, a cDNA clone of the rat c-myb message, referred to as pc8myb, was used. The competitor RNA comprises a deletion of 50 bases, making it smaller than the wild-type cellular RNA, and spansfrom nucleotide 428 to nucleotide 753.

A house-keeping gene, GAPDH, that is constitutively expressed by the RASMC, was used as an internal control for QCPCR assay. A deletion competitor and wild-type controls for GAPDH were made the same way as for *c-myb*. GAPDH-containing plasmid (pTri-GAPDH) was purchased from Ambion. The GAPDH competitor is also a deletion mutant, lacking 50 bases. The GAPDH competitor was used to quantitate the amount of this housekeeping gene in each

sample, thus allowing for a confirmation of cellular RNA's integrity and for the efficiency of RNA isolation. All quantitations for the level of *c-myb* expression were normalized to the level of GAPDH expression in the same sample of cells.

Referring to Fig. 19, RASMC that were treated with a stabilized catalytically active 575 HH ribozyme did not proliferate well. There was greater than 70 % inhibition of RASMC proliferation when compared with approximately 25% inhibition of cell proliferation by a catalytically inactive version of the 575 HH ribozyme. The level of inhibition of RASMC proliferation correlates very well with the greater than 70 % decrease in the level of *c-myb* RNA. This shows that the inhibition of smooth muscle cell proliferation is directly mediated by the cleavage of *c-myb* RNA by a ribozyme in RASMC.

Figure 20 shows what Applicant presently believes is an optimal ribozyme configuration

Example 26: Inhibition of smooth muscle cell proliferation by 2-5A antisense chimera.

By "2-5A antisense chimera" is meant, an antisense oligonucleotide containing a 5' phosphorylated 2'-5'-linked adenylate residues. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which in turn cleaves the target RNA (Torrence et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300).

RNAs containing 2'-5' Adenosine with a terminal 5' phosphate has been shown to activate RNAse L (Torrence et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300). The terminal phosphate is required for efficient activation of RNAse L. Ribozymes targeting *c-myb* site 575 were synthesized with 2-5A moieties on the 5' end, with and without the terminal 5' phosphate. The ribozyme-2-5A chimera was complexed with LipofectAMINE and assayed on rat aortic smooth muscle cells (RASMC) as described above.

As shown in Figure 21, when no terminal phosphate is present, the active ribozyme [575 inactive Rz+ inactive (A)4] functions similarly to a normal active ribozyme lacking a 2-5A modification (575 active Rz). An inactive ribozyme core with 5' phosphate-2-5A [575 inactive Rz+ active P(A)4] shows significant inhibition relative to the controls, but has significantly lower activity when compared with an active ribozyme. A molecule that contains both an active ribozyme core and 5' phosphate-contining 2-5A [575 active Rz+active P (A)4] shows even greater inhibition than that obtained by either mechanism individually, inhibiting the smooth muscle cell proliferation to baseline levels (0% FBS). Thus the ribozyme and 2-5A anitisense chimera together show an additive effect in inhibiting RASMC proliferation.

<u>Use of Ribozymes That Cleave c-*myb* RNA to Treat Restenosis.</u>

The above discussion demonstrates, by way of example, how ribozymes that inhibit smooth muscle cell proliferation are delivered directly, or through the use of expression vectors, to vessels. Preferably, ribozymes cleaving c-*myb* RNA are delivered to vessels at the time of coronary angioplasty. Local delivery during intervention can be achieved through the use of double balloon catheters , porous balloon catheters , balloon catheters coated with polymers (Riessen, R., et al., 1993, *Human Gene Therapy*, 4, 749-758), or biopolymer stents (Slepian and Schindler, U.S. Patent # 5,213,580). In the above examples, ribozymes were identified that could inhibit roughly half of the smooth muscle cells in culture from proliferating in response to the growth factors present in serum. A corresponding 50% (or even lower) reduction in intimal thickening will significantly improve the outcome of patients undergoing coronary angioplasty.

Use of Ribozymes Targeting *c-myb* to Treat Cancer

Overexpression of the *c-myb* oncogene has been reported in a number of cancers, including leukemias, neuroblastomas, and lung, colon, and breast carcinomas (Torelli, G., et al., 1987, *Cancer Res.*, 47, 5266-5269; Slamon, D. J., et al., 1986, *Science*, 233, 203-206; Slamon, D. J., et al., 1984, *Science*, 224, 256-262; Thiele, C. J., et al., 1988, *Mol. Cell. Biol.*, 8, 1677-1683; Griffin, C. A. and Baylin, S. B., 1985, *Cancer Res.*, 45, 272-275; Alitalo, K., et al., 1984, *Proc. Natl. Acad. Sci. USA*, 81, 4534-4538). Thus, inhibition of *c-myb* expression can reduce cell proliferation of a number of cancers. Indeed, in tissue culture, treatment of colon adenocarcinoma, neurectodermal, and myeloid leukemia cell lines with antisense *c-myb* oligonucleotides inhibits their proliferation (Melani, C., et al., 1991, *Cancer Res.*, 51, 2897-2901; Raschella, F., et al., 1992, *Cancer Res.*, 52, 4221-4226; Anfossi, G., et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86, 3379-3383). Furthermore, myeloid cells from patients with chronic myelogenous leukemia and acute myelogenous leukemia are differentially sensitive to *c-myb* antisense oligonucleotides (Calabretta, B., et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88, 2351-2355). Ratajczak, et al. (1992, *Proc. Natl. Acad. Sci. USA*, 89, 11823-11827) treated mice bearing human leukemia cells with *c-myb* antisense oligonucleotides and significantly prolonged their survival and reduced their tumor burden. Thus, reduction of *c-myb* expression in leukemic cells in tissue culture and *in vivo* can reduce their proliferative potential.

While the above studies demonstrated that antisense oligonucleotides can efficiently reduce the expression of *c-myb* in cancer cells and reduce their ability to proliferate and spread, this invention describes enzymatic RNAs, or ribozymes, shown to cleave *c-myb* RNA. Such ribozymes, with their catalytic activity and increased site specificity (see above), are likely to represent more

potent and safe therapeutic molecules than antisense oligonucleotides for the treatment of cancer as well as restenosis. In the present invention, ribozymes are shown to inhibit smooth muscle cell proliferation. From those practiced in the art, it is clear from the examples described, that the same ribozymes may be delivered in a similar fashion to cancer cells to block their proliferation.

In a preferred embodiment, autologous bone marrow from patients suffering with acute myelogenous leukemia or chronic myelogenous leukemia are treated with ribozymes that cleave c-*myb* RNA. Ribozymes will be delivered to the autologous bone marrow cells *ex vivo* at 0.1 to 50 μM with or without forming complexes of the ribozymes with cationic lipids, encapsulating in liposomes or alternative delivery agents. After several days, the proliferative capacity of the leukemic cells in the patients bone marrow will be reduced. The patient's endogenous bone marrow cells will be depleted by chemical or radiation treatments and their bone marrow reconstituted with the *ex vivo* treated cells. In such autologous bone marrow reconstitution treatments of leukemic patients, recurrence of the disease can be caused by proliferation of leukemic cells present in the transplanted bone marrow. Significantly reducing the proliferative potential of the leukemic cells by treating with ribozymes that cleave c-*myb* RNA will reduce the risk of recurrent leukemia.

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of *c-myb* RNA in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this

invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other in vitro uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with *c-myb*_related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a

polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, *c-myb*) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Other embodiments are within the following claims.

Table I: Characteristics of Ribozymes

Group I Introns

Size: ~200 to >1000 nucleotides

Requires a U in the target sequence immediately 5' of the cleavage site

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in Tetrahymena thermophila rRNA, fungal mitochondria, chloroplasts, phage T4, blue-gree algae, and others.

RNAseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group are all bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figure 1)

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50-60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

Neurospora VS RNA Ribozyme

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in Neurospora VS RNA (Figure 5).

Table II: Ribozyme catalyzed cleavage of c-myb RNA

Hammerhead Sites			% Cleavage	
Cleavage Site	Sequence ID No.	Target Sequence	Mouse *c-myb* RNA	Human *c-myb* RNA
310	79	CGUCACU U GGGGAAA	28.5	0.1
549	80	GUCUGUU A UUGCCAA	87.4	91.6
551	81	CUGUUAU U GCCAAGC	56.8	82.4
575	82	GGAGAAU U GGAAAAC	93.9	91.3
634	83	AAAACCU C CUGGACA	68.4	87.1
738	84	UAAUGCU A UCAAGAA	78.1	0.01
839	85	CAAGCUU C CAGAAGA	27.2	0.01
936	86	UUCCUAU U ACCACAU	61.8	60.6
1017	87	UGUCCCU C AGCCAGC	40.3	0.1
1082	88	AGCGAAU A AAGGAAU	55.2	89.2
1363	89	UUAGAAU U UGCAGAA	11.6	0.1
1553	90	CAGCUAU C AAAAGGU	87.1	92.5
1597	91	ACACCAU U CAAACAU	71.2	62.7
1598	92	CACCAUU C AAACAUG	79.6	85.5

Hammerhead Sites			% Cleavage	
Cleavage Site	Sequence ID No.	Target Sequence	Mouse c-*myb* RNA	Human c-*myb* RNA
1635	93	AUACGGU C CCCUGAA	84.4	82.3
1721	94	CUGGAAU U GUUGCUG	62.1	79.3
1724	95	GAAUUGU U GCUGAGU	65.6	86
1895	96	AUAUUCU U ACAAGCU	79.1	66.2
1909	97	UCCGUUU U AAUGGCA	31.1	0.1
1943	98	ACAAUGU U CUCAAAG	66.1	80
Hairpin Ribozymes				
1632	99	ACG GUCC CCUGAAG	92.8	84.6
2231	100	ACA GUUG AGAGCAG	0.1	0.1

[a] The nucleotide numbers given correspond to the nucleotide just 5′ of the ribozyme cleavage site in the human c-myb sequence taken from Westin, et al., supra (GenBank Accession No. X52125). All but two of the sequences (310; I.D. No. 79 and 2231; I. D. No. 100) overlap sequences in Table I.

Table III: Sequences of ribozymes used in these studies.

Target Site	Sequence ID No.	Ribozyme Sequence
Hammerhead ribozymes with 7 nucleotide binding arms		
310	101	UUUCCCCCUGAUGAGGCCGAAAGGCCGAAAGUGACG
549	102	UUGGCAACUGAUGAGGCCGAAAGGCCGAAAACAGAC
551	103	GCUUGGCCUGAUGAGGCCGAAAGGCCGAAAUAACAG
575	104	GCUUUCCCUGAUGAGGCCGAAAGGCCGAAAUUCUCC
634	105	UGUCCAGCUGAUGAGGCCGAAAGGCCGAAAGGUUUU
738	106	UUCUUGACUGAUGAGGCCGAAAGGCCGAAAGCAUUA
839	107	UCUUCUGCUGAUGAGGCCGAAAGGCCGAAAAGCUCG
936	108	AUGUGGUCUGAUGAGGCCGAAAGGCCGAAAUAGGAA
1017	109	GCCGGCUCUGAUGAGCGCGAAAGCGCGAAAGGGACG
1082	110	GCUCCUUCUGAUGAGGCCGAAAGGCCGAAAUUCGCU
1363	111	UUCUGCACUGAUGAGGCCGAAAGGCCGAAAUUCUAA

1553	112	ACCUUUUCUGAUGAGGCCGAAAGGCCGAAAUAGCUG
1597	113	AUGUUUGCUGAUGAGGCCGAAAGGCCGAAAUGGUGU
1598	114	CAUGUUUCUGAUGAGGCCGAAAGGCCGAAAAUGGUG
1635	115	UUCAGGGCUGAUGAGGCCGAAAGGCCGAAACCGUAU
1721	116	CAGCAACCUGAUGAGGCCGAAAGGCCGAAAUUCCAG
1724	117	ACUCAGCCUGAUGAGGCCGAAAGGCCGAAACAAUUC
1895	118	AGCUUGUCUGAUGAGGCCGAAAGGCCGAAAGAAUAU
1909	119	UGUCAUUCUGAUGAGGCCGAAAGGCCGAAAAACAGA
1943	120	CUUUGAGCUGAUGAGGCCGAAAGGCCGAAACAUUGU
		Bimolecular Hairpin Ribozymes
1632[a]	121	5' Fragment: UCAGGGAGAAGUAUACCAGAGAAACACACGCG 3' Fragment: CGCGUGGUACAUUACCUGGUA
2231[a]	122	5' Fragment: GCUCUCAGAAGUUGACCAGAGAAACACACGCG 3' Fragment: CGCGUGGUACAUUACCUGGUA
Hammerhead riboyzmes with 6, 8, 9, 10, and 12 nucleotide binding arms		
575 6/6[b]	123	CUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUC
575 8/8	124	UGCUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUCCC
575 9/9	125	CUGCUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUCCCU
575 10/10	126	ACUGCUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUCCCUU
575 12/12	127	ACACUGCUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUCCCUUUU
549 12/12	128	AGUGCUUGGCAACUGAUGAGGCCGAAAGGCCGAA AACAGACCAACG
1553 12/12	129	GAUUGACCUUUUCUGAUGAGGCCGAAAGGCCGAA AUAGCUGGAGUU

[a]The hairpin ribozymes were synthesized in two pieces as indicated. The two oligonucleotides were annealed and tested for activity against the c-myb RNA as described above. See Mamone, Ribozyme synthesis, filed May 11,

1992, U.S.S.N. 07/882,689, hereby incorporated by reference herein.

[b]Designation of the ribozymes with different arm lengths is a/b where (a) represents the nucleotides in stem I and (b) represents the nucleotides in stem III (see Figure 1).

Table IV: Comparison of the effects six hammerhead ribozymes, that cleave *c-myb* RNA, on smooth muscle cell proliferation

	Inactive Ribozyme	Active Ribozyme	% Inhibition
Ribozyme Site	% Cell Proliferation	% Cell Proliferation	(Active vs. Inactive)
549	68 ± 1	59.5 ± 1.5	14 ± 4
575	66.5 ± 0.5	54.5 ± 1.5	21 ± 3
1553	68.5 ± 0.5	52 ± 1	28 ± 1
1597	66 ± 1	57 ± 3	16 ± 7
1598	67 ± 1	58.5 ± 0.5	15 ± 1
1635	62.5 ± 2.5	64 ± 1	0

Table V: Dose Response of *c-myb* Hairpin Ribozyme 1632

	Control Ribozyme	Ribozyme 1632	
Ribozyme Dose (μM)	% Proliferation	% Proliferation	% Inhibition (vs. control)
0.05	86.5 ± 1.5	88 ± 5	0
0.15	89.5 ± 1.5	78.5 ± 2.5	10 ± 5
0.45	87.5 ± 1	66.5 ± 1.5	25 ± 4

Table VI: Dose Response of *c-myb* Hammerhead Ribozymes 575 and 549

	Control Ribozyme	Ribozyme 575		Ribozyme 549	
Ribozyme Dose (μM)	% cells in S phase	% cells in S phase	% Inhibition (vs. control)	% cells in S phase	% Inhibition (vs. control)
0.05	89±5	77.5±1.5	14±8	92±1	0
0.15	90±1	68.5±1.5	26±2	84±2	9±4
0.45	91.5±0.5	59±5	38±7	76.5±2.5	18±5

Table VII: Delivery of *c-myb* Ribozyme 575 by Two Different Cationic Lipids

Delivery with DMRIE/DOPE			
	Inactive Ribozyme 575	Active Ribozyme 575	
Ribozyme Dose (μM)	% cells in S phase	% cells in S phase	% Inhibition (vs. inactive)
0.075	79 ± 6	74.5 ± 1.5	6 ± 6
0.15	79.5 ± 0.5	67 ± 1	17 ± 4
0.30	77 ± 1	57 ± 2	28 ± 5
Delivery with Lipofectamine			
	Inactive Ribozyme 575	Active Ribozyme 575	
Ribozyme Dose (μM)	% cells in S phase	% cells in S phase	% Inhibition (vs. inactive)
0.075	81 ± 1	83 ± 1	0
0.15	79 ± 3	71 ± 1	11 ± 4
0.30	82 ± 1	68.5 ± 1.5	18 ± 4
0.60	75 ± 1	59.5 ± 3.5	22 ± 7

Table VIII: Arm Length Variations of *c-myb* Hammerhead Ribozyme 575

Arm Length (base pairs)	% cells in S phase	% Inhibition (vs. Inactive 7/7)
6/6	62 ± 1	4 ± 4
7/7	60 ± 1	7 ± 3
8/8	60.5 ± 0.5	6 ± 2
9/9	53.5 ± 0.5	18 ± 2
10/10	55 ± 1	16 ± 4
12/12	48 ± 1	28 ± 3

Table IX: Hammerhead ribozymes with 7 vs. 12-nucleotide binding arms targeting three different sites

Ribozyme Target Site	Length of Binding Arms	Inactive Ribozyme (% Cell Prolifera-tion)	Active Ribozyme (% Cell Prolifera-tion)	% Inhibition (Active vs. Inactive)
575	7/7	51.5 ± 0.5	43 ± 0.5	24 ± 5
575	12/12	50.5 ± 3.5	37 ± 0.5	37 ± 4
549	7/7	49.5 ± 0.5	44.5 ± 1.5	21 ± 7
549	12/12	48.5 ± 1.5	35 ± 2	41 ± 7
1553	7/7	49.5 ± 0.5	43.5 ± 2.5	23 ± 9
1553	12/12	49 ± 1	33.5 ± 1.5	45 ± 6

Table X: Effect of chloroquine on ribozyme inhibition of smooth muscle cell proliferation

Ribozyme	Chloro-quine (μM)	Inactive Ribozyme (% Cell Prolifera-tion)	Active Ribozyme (% Cell Prolifera-tion)	% Inhibi-tion (Active vs. inactive)
575, 12/12	0	81.8 ± 0.5	74 ± 1	10 ± 2
575, 12/12	10	83 ± 4	62.5 ± 0.5	28 ± 6

Table XI: Inhibition of Human Aortic Smooth Muscle Cells by *c-myb* Ribozyme 549

	Inactive Ribozyme	Active Ribozyme	% Inhibition
Ribozyme Dose (μM)	% Proliferation	% Proliferation	(active vs. inactive)
0.075	55 ± 2	40.5 ± 4.5	30 ± 13
0.15	53 ± 10	42 ± 1	23 ± 23
0.30	53 ± 7	32.5 ± 4.5	44 ± 22

Table XII: Inhibition of Rat Smooth Muscle Cell Proliferation by Direct Addition of a Chemically-Modified *c-myb* Ribozyme 575

	Inactive Ribozyme	Active Ribozyme	% Inhibition
Ribozyme Dose (μM)	% Proliferation	% Proliferation	(active vs. inactive)
0.22	42 ± 3	36 ± 0.5	15 ± 8
0.67	48 ± 3	35 ± 2	28 ± 9
2.0	52 ± 5	25 ± 1	54 ± 7

Table XIII: Human c-myb Hairpin Ribozyme and Target Sequences

Position	Ribozyme Sequence	Target
104	CCCUCCCC AGAA GCGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCGCA GCC GGGGAGGG
148	ACCGACCG AGAA GCCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGGCA GCC CGGUCGGU
185	GCGCGGCG AGAA GCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCGCC GCC CGCCGCGC
528	ACGUUUCG AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUACG GUC CGAAACGU

Posi-tion	Ribozyme Sequence	Target
715	UUCGUCCA AGAA GUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUACU GCC UGGACGAA
1025	AUGGCUGC AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGCU GCC GCAGCCAU
1187	CUGGUGUG AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUGCC GAC CACACCAG
1532	GUUCUAAA AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUACU GUU UUUAGAAC
1632	CUUCAGGG AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUACG GUC CCCUGAAG
1836	GGUAUUCA AGAA GUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGACA GUC UGAAUACC
1852	UCUGCGUG AGAA GUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAACU GUU CACGCAGA
1861	CAGGCGAG AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACGCA GAC CUCGCCUG
1993	UGCUACAA AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUGCA GCC UUGUAGCA
2231	CUGCUCUC AGAA GUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAACA GUU GAGAGCAG
2316	UUAGGUAA AGAA GUUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UAACA GUC UUACCUAA
3068	AAUUAUAA AGAA GUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGACU GUU UUAUAAUU
3138	AUCCAUGC AGAA GUUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAACU GUU GCAUGGAU
3199	GUUCUUAA AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCACU GCC UUAAGAAC
3264	UGCUACAA AGAA GUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUACU GCC UUGUAGCA

Table XIV: Human c-myb Hammerhead Ribozyme and Target Sequence

nt. Position	Target Sequence	Ribozyme Sequence
14	CAACCUGU U UCCUCCUC	GAGGAGGA CUGAUGA X GAA ACAGGUUG
15	AACCUGUU U CCUCCUCC	GGAGGAGG CUGAUGA X GAA AACAGGUU
16	ACCUGUUU C CUCCUCCU	AGGAGGAG CUGAUGA X GAA AAACAGGU
19	UGUUUCCU C CUCCUCCU	AGGAGGAG CUGAUGA X GAA AGGAAACA
22	UUCCUCCU C CUCCUUCU	AGAAGGAG CUGAUGA X GAA AGGAGGAA
25	CUCCUCCU C CUUCUCCU	AGGAGAAG CUGAUGA X GAA AGGAGGAG
28	CUCCUCCU U CUCCUCCU	AGGAGGAG CUGAUGA X GAA AGGAGGAG
29	UCCUCCUU C UCCUCCUC	GAGGAGGA CUGAUGA X GAA AAGGAGGA
31	CUCCUUCU C CUCCUCCU	AGGAGGAG CUGAUGA X GAA AGAAGGAG
34	CUUCUCCU C CUCCUCCG	CGGAGGAG CUGAUGA X GAA AGGAGAAG
37	CUCCUCCU C CUCCGUGA	UCACGGAG CUGAUGA X GAA AGGAGGAG
40	CUCCUCCU C CGUGACCU	AGGUCACG CUGAUGA X GAA AGGAGGAG
49	CGUGACCU C CUCCUCCU	AGGAGGAG CUGAUGA X GAA AGGUCACG
52	GACCUCCU C CUCCUCUU	AAGAGGAG CUGAUGA X GAA AGGAGGUC
55	CUCCUCCU C CUCUUUCU	AGAAAGAG CUGAUGA X GAA AGGAGGAG
58	CUCCUCCU C UUUCUCCU	AGGAGAAA CUGAUGA X GAA AGGAGGAG
60	CCUCCUCU U UCUCCUGA	UCAGGAGA CUGAUGA X GAA AGAGGAGG
61	CUCCUCUU U CUCCUGAG	CUCAGGAG CUGAUGA X GAA AAGAGGAG
62	UCCUCUUU C UCCUGAGA	UCUCAGGA CUGAUGA X GAA AAAGAGGA
64	CUCUUUCU C CUGAGAAA	UUUCUCAG CUGAUGA X GAA AGAAAGAG
75	GAGAAACU U CGCCCCAG	CUGGGGCG CUGAUGA X GAA AGUUUCUC
76	AGAAACUU C GCCCCAGC	GCUGGGGC CUGAUGA X GAA AAGUUUCU
156	AGCCCGGU C GGUCCCCG	CGGGGACC CUGAUGA X GAA ACCGGGCU
160	CGGUCGGU C CCCGCGGC	GCCGCGGG CUGAUGA X GAA ACCGACCG
170	CCGCGGCU C UCGCGGAG	CUCCGCGA CUGAUGA X GAA AGCCGCGG
172	GCGGCUCU C GCGGAGCC	GGCUCCGC CUGAUGA X GAA AGAGCCGC
224	CACAGCAU A UAUAGCAG	CUGCUAUA CUGAUGA X GAA AUGCUGUG
226	CAGCAUAU A UAGCAGUG	CACUGCUA CUGAUGA X GAA AUAUGCUG
228	GCAUAUAU A GCAGUGAC	GUCACUGC CUGAUGA X GAA AUAUAUGC
253	UGAGGACU U UGAGAUGU	ACAUCUCA CUGAUGA X GAA AGUCCUCA
254	GAGGACUU U GAGAUGUG	CACAUCUC CUGAUGA X GAA AAGUCCUC
274	CCAUGACU A UGAUGGGC	GCCCAUCA CUGAUGA X GAA AGUCAUGG
287	GGGCUGCU U CCCAAGUC	GACUUGGG CUGAUGA X GAA AGCAGCCC
288	GGCUGCUU C CCAAGUCU	AGACUUGG CUGAUGA X GAA AAGCAGCC

nt. Position	Target Sequence	Ribozyme Sequence
295	UCCCAAGU C UGGAAAGC	GCUUUCCA CUGAUGA X GAA ACUUGGGA
306	GAAAGCGU C ACUUGGGG	CCCCAAGU CUGAUGA X GAA ACGCUUUC
310	GCGUCACU U GGGGAAAA	UUUUCCCC CUGAUGA X GAA AGUGACGC
392	UGGAAAGU U AUUGCCAA	UUGGCAAU CUGAUGA X GAA ACUUUCCA
393	GGAAAGUU A UUGCCAAU	AUUGGCAA CUGAUGA X GAA AACUUUCC
395	AAAGUUAU U GCCAAUUA	UAAUUGGC CUGAUGA X GAA AUAACUUU
402	UUGCCAAU U AUCUCCCG	CGGGAGAU CUGAUGA X GAA AUUGGCAA
403	UGCCAAUU A UCUCCCGA	UCGGGAGA CUGAUGA X GAA AAUUGGCA
405	CCAAUUAU C UCCCGAAU	AUUCGGGA CUGAUGA X GAA AUAAUUGG
414	UCCCGAAU C GAACAGAU	AUCUGUUC CUGAUGA X GAA AUUCGGGA
452	CAGAAAGU A CUAAACCC	GGGUUUAG CUGAUGA X GAA ACUUUCUG
455	AAAGUACU A AACCCUGA	UCAGGGUU CUGAUGA X GAA AGUACUUU
467	CCUGAGCU C AUCAAGGG	CCCUUGAU CUGAUGA X GAA AGCUCAGG
470	GAGCUCAU C AAGGGUCC	GGACCCUU CUGAUGA X GAA AUGAGCUC
477	UCAAGGGU C CUUGGACC	GGUCCAAG CUGAUGA X GAA ACCCUUGA
480	AGGGUCCU U GGACCAAA	UUUGGUCC CUGAUGA X GAA AGGACCCU
498	AAGAAGAU C AGAGAGUG	CACUCUCU CUGAUGA X GAA AUCUUCUU
509	AGAGUGAU A GAGCUUGU	ACAAGCUC CUGAUGA X GAA AUCACUCU
515	AUAGAGCU U GUACAGAA	UUCUGUAC CUGAUGA X GAA AGCUCUAU
518	GAGCUUGU A CAGAAAUA	UAUUUCUG CUGAUGA X GAA ACAAGCUC
526	ACAGAAAU A CGGUCCGA	UCGGACCG CUGAUGA X GAA AUUUCUGU
531	AAUACGGU C CGAAACGU	ACGUUUCG CUGAUGA X GAA ACCGUAUU
540	CGAAACGU U GGUCUGUU	AACAGACC CUGAUGA X GAA ACGUUUCG
544	ACGUUGGU C UGUUAUUG	CAAUAACA CUGAUGA X GAA ACCAACGU
548	UGGUCUGU U AUUGCCAA	UUGGCAAU CUGAUGA X GAA ACAGACCA
549	GGUCUGUU A UUGCCAAG	CUUGGCAA CUGAUGA X GAA AACAGACC
551	UCUGUUAU U GCCAAGCA	UGCUUGGC CUGAUGA X GAA AUAACAGA
562	CAAGCACU U AAAGGGGA	UCCCCUUU CUGAUGA X GAA AGUGCUUG
563	AAGCACUU A AAGGGGAG	CUCCCCUU CUGAUGA X GAA AAGUGCUU
575	GGGAGAAU U GGAAAACA	UGUUUUCC CUGAUGA X GAA AUUCUCCC
588	AACAAUGU A GGGAGAGG	CCUCUCCC CUGAUGA X GAA ACAUUGUU
603	GGUGGCAU A ACCACUUG	CAAGUGGU CUGAUGA X GAA AUGCCACC
615	ACUUGAAU C CAGAAGUU	AACUUCUG CUGAUGA X GAA AUUCAAGU
623	CCAGAAGU U AAGAAAAC	GUUUUCUU CUGAUGA X GAA ACUUCUGG
624	CAGAAGUU A AGAAAACC	GGUUUUCU CUGAUGA X GAA AACUUCUG
634	GAAAACCU C CUGGACAG	CUGUCCAG CUGAUGA X GAA AGGUUUUC

nt. Position	Target Sequence	Ribozyme Sequence
659	GACAGAAU U AUUUACCA	UGGUAAAU CUGAUGA X GAA AUUCUGUC
660	ACAGAAUU A UUUACCAG	CUGGUAAA CUGAUGA X GAA AAUUCUGU
662	AGAAUUAU U UACCAGGC	GCCUGGUA CUGAUGA X GAA AUAAUUCU
663	GAAUUAUU U ACCAGGCA	UGCCUGGU CUGAUGA X GAA AAUAAUUC
664	AAUUAUUU A CCAGGCAC	GUGCCUGG CUGAUGA X GAA AAAUAAUU
704	GCAGAAAU C GCAAAGCU	AGCUUUGC CUGAUGA X GAA AUUUCUGC
713	GCAAAGCU A CUGCCUGG	CCAGGCAG CUGAUGA X GAA AGCUUUGC
732	GAACUGAU A AUGCUAUC	GAUAGCAU CUGAUGA X GAA AUCAGUUC
738	AUAAUGCU A UCAAGAAC	GUUCUUGA CUGAUGA X GAA AGCAUUAU
740	AAUGCUAU C AAGAACCA	UGGUUCUU CUGAUGA X GAA AUAGCAUU
756	ACUGGAAU U CUACAAUG	CAUUGUAG CUGAUGA X GAA AUUCCAGU
757	CUGGAAUU C UACAAUGC	GCAUUGUA CUGAUGA X GAA AAUUCCAG
759	GGAAUUCU A CAAUGCGU	ACGCAUUG CUGAUGA X GAA AGAAUUCC
768	CAAUGCGU C GGAAGGUC	GACCUUCC CUGAUGA X GAA ACGCAUUG
776	CGGAAGGU C GAACAGGA	UCCUGUUC CUGAUGA X GAA ACCUUCCG
789	AGGAAGGU U AUCUGCAG	CUGCAGAU CUGAUGA X GAA ACCUUCCU
790	GGAAGGUU A UCUGCAGG	CCUGCAGA CUGAUGA X GAA AACCUUCC
792	AAGGUUAU C UGCAGGAG	CUCCUGCA CUGAUGA X GAA AUAACCUU
802	GCAGGAGU C UUCAAAAG	CUUUUGAA CUGAUGA X GAA ACUCCUGC
804	AGGAGUCU U CAAAAGCC	GGCUUUUG CUGAUGA X GAA AGACUCCU
805	GGAGUCUU C AAAAGCCA	UGGCUUUU CUGAUGA X GAA AAGACUCC
838	CACAAGCU U CCAGAAGA	UCUUCUGG CUGAUGA X GAA AGCUUGUG
839	ACAAGCUU C CAGAAGAA	UUCUUCUG CUGAUGA X GAA AAGCUUGU
852	AGAACAGU C AUUUGAUG	CAUCAAAU CUGAUGA X GAA ACUGUUCU
855	ACAGUCAU U UGAUGGGU	ACCCAUCA CUGAUGA X GAA AUGACUGU
856	CAGUCAUU U GAUGGGUU	AACCCAUC CUGAUGA X GAA AAUGACUG
864	UGAUGGGU U UUGCUCAG	CUGAGCAA CUGAUGA X GAA ACCCAUCA
865	GAUGGGUU U UGCUCAGG	CCUGAGCA CUGAUGA X GAA AACCCAUC
866	AUGGGUUU U GCUCAGGC	GCCUGAGC CUGAUGA X GAA AAACCCAU
870	GUUUUGCU C AGGCUCCG	CGGAGCCU CUGAUGA X GAA AGCAAAAC
876	CUCAGGCU C CGCCUACA	UGUAGGCG CUGAUGA X GAA AGCCUGAG
882	CUCCGCCU A CAGCUCAA	UUGAGCUG CUGAUGA X GAA AGGCGGAG
888	CUACAGCU C AACUCCCU	AGGGAGUU CUGAUGA X GAA AGCUGUAG
893	GCUCAACU C CCUGCCAC	GUGGCAGG CUGAUGA X GAA AGUUGAGC
917	CCCACUGU U AACAACGA	UCGUUGUU CUGAUGA X GAA ACAGUGGG
928	CAACGACU A UUCCUAUU	AAUAGGAA CUGAUGA X GAA AGUCGUUG

nt. Position	Target Sequence	Ribozyme Sequence
930	ACGACUAU U CCUAUUAC	GUAAUAGG CUGAUGA X GAA AUAGUCGU
931	CGACUAUU C CUAUUACC	GGUAAUAG CUGAUGA X GAA AAUAGUCG
934	CUAUUCCU A UUACCACA	UGUGGUAA CUGAUGA X GAA AGGAAUAG
936	AUUCCUAU U ACCACAUU	AAUGUGGU CUGAUGA X GAA AUAGGAAU
937	UUCCUAUU A CCACAUUU	AAAUGUGG CUGAUGA X GAA AAUAGGAA
944	UACCACAU U UCUGAAGC	GCUUCAGA CUGAUGA X GAA AUGUGGUA
945	ACCACAUU U CUGAAGCA	UGCUUCAG CUGAUGA X GAA AAUGUGGU
946	CCACAUUU C UGAAGCAC	GUGCUUCA CUGAUGA X GAA AAAUGUGG
962	CAAAAUGU C UCCAGUCA	UGACUGGA CUGAUGA X GAA ACAUUUUG
964	AAAUGUCU C CAGUCAUG	CAUGACUG CUGAUGA X GAA AGACAUUU
969	UCUCCAGU C AUGUUCCA	UGGAACAU CUGAUGA X GAA ACUGGAGA
974	AGUCAUGU U CCAUACCC	GGGUAUGG CUGAUGA X GAA ACAUGACU
975	GUCAUGUU C CAUACCCU	AGGGUAUG CUGAUGA X GAA AACAUGAC
979	UGUUCCAU A CCCUGUAG	CUACAGGG CUGAUGA X GAA AUGGAACA
986	UACCCUGU A GCGUUACA	UGUAACGC CUGAUGA X GAA ACAGGGUA
991	UGUAGCGU U ACAUGUAA	UUACAUGU CUGAUGA X GAA ACGCUACA
992	GUAGCGUU A CAUGUAAA	UUUACAUG CUGAUGA X GAA AACGCUAC
1002	AUGUAAAU A UAGUCAAU	AUUGACUA CUGAUGA X GAA AUUUACAU
1004	GUAAAUAU A GUCAAUGU	ACAUUGAC CUGAUGA X GAA AUAUUUAC
1007	AAUAUAGU C AAUGUCCC	GGACAUU CUGAUGA X GAA ACUAUAUU
1013	GUCAAUGU C CCUCAGCC	GGCUGAGG CUGAUGA X GAA ACAUUGAC
1017	AUGUCCCU C AGCCAGCU	AGCUGGCU CUGAUGA X GAA AGGGACAU
1037	GCAGCCAU U CAGAGACA	UGUCUCUG CUGAUGA X GAA AUGGCUGC
1048	GAGACACU A UAAUGAUG	CAUCAUUA CUGAUGA X GAA AGUGUCUC
1050	GACACUAU A AUGAUGAA	UUCAUCAU CUGAUGA X GAA AUAGUGUC
1082	AAGCGAAU A AAGGAAUU	AAUUCCUU CUGAUGA X GAA AUUCGCUU
1090	AAAGGAAU U AGAAUUGC	GCAAUUCU CUGAUGA X GAA AUUCCUUU
1091	AAGGAAUU A GAAUUGCU	AGCAAUUC CUGAUGA X GAA AAUUCCUU
1096	AUUAGAAU U GCUCCUAA	UUAGGAGC CUGAUGA X GAA AUUCUAAU
1100	GAAUUGCU C CUAAUGUC	GACAUUAG CUGAUGA X GAA AGCAAUUC
1103	UUGCUCCU A AUGUCAAC	GUUGACAU CUGAUGA X GAA AGGAGCAA
1108	CCUAAUGU C AACCGAGA	UCUCGGUU CUGAUGA X GAA ACAUUAGG
1124	AAUGAGCU A AAAGGACA	UGUCCUUU CUGAUGA X GAA AGCUCAUU
1184	ACCACCAU U GCCGACCA	UGGUCGGC CUGAUGA X GAA AUGGUGGU
1203	CCAGACCU C AUGGAGAC	GUCUCCAU CUGAUGA X GAA AGGUCUGG
1223	GCACCUGU U UCCUGUUU	AAACAGGA CUGAUGA X GAA ACAGGUGC

nt. Position	Target Sequence	Ribozyme Sequence
1224	CACCUGUU U CCUGUUUG	CAAACAGG CUGAUGA X GAA AACAGGUG
1225	ACCUGUUU C CUGUUUGG	CCAAACAG CUGAUGA X GAA AAACAGGU
1230	UUUCCUGU U UGGGAGAA	UUCUCCCA CUGAUGA X GAA ACAGGAAA
1231	UUCCUGUU U GGGAGAAC	GUUCUCCC CUGAUGA X GAA AACAGGAA
1246	ACACCACU C CACUCCAU	AUGGAGUG CUGAUGA X GAA AGUGGUGU
1251	ACUCCACU C CAUCUCUG	CAGAGAUG CUGAUGA X GAA AGUGGAGU
1255	CACUCCAU C UCUGCCAG	CUGGCAGA CUGAUGA X GAA AUGGAGUG
1257	CUCCAUCU C UGCCAGCG	CGCUGGCA CUGAUGA X GAA AGAUGGAG
1269	CAGCGGAU C CUGGCUCC	GGAGCCAG CUGAUGA X GAA AUCCGCUG
1276	UCCUGGCU C CCUACCUG	CAGGUAGG CUGAUGA X GAA AGCCAGGA
1280	GGCUCCCU A CCUGAAGA	UCUUCAGG CUGAUGA X GAA AGGGAGCC
1297	AAGCGCCU C GCCAGCAA	UUGCUGGC CUGAUGA X GAA AGGCGCUU
1316	UGCAUGAU C GUCCACCA	UGGUGGAC CUGAUGA X GAA AUCAUGCA
1319	AUGAUCGU C CACCAGGG	CCCUGGUG CUGAUGA X GAA ACGAUCAU
1334	GGCACCAU U CUGGAUAA	UUAUCCAG CUGAUGA X GAA AUGGUGCC
1335	GCACCAUU C UGGAUAAU	AUUAUCCA CUGAUGA X GAA AAUGGUGC
1341	UUCUGGAU A AUGUUAAG	CUUAACAU CUGAUGA X GAA AUCCAGAA
1346	GAUAAUGU U AAGAACCU	AGGUUCUU CUGAUGA X GAA ACAUUAUC
1347	AUAAUGUU A AGAACCUC	GAGGUUCU CUGAUGA X GAA AACAUUAU
1355	AAGAACCU C UUAGAAUU	AAUUCUAA CUGAUGA X GAA AGGUUCUU
1357	GAACCUCU U AGAAUUUG	CAAAUUCU CUGAUGA X GAA AGAGGUUC
1358	AACCUCUU A GAAUUUGC	GCAAAUUC CUGAUGA X GAA AAGAGGUU
1363	CUUAGAAU U UGCAGAAA	UUUCUGCA CUGAUGA X GAA AUUCUAAG
1364	UUAGAAUU U GCAGAAAC	GUUUCUGC CUGAUGA X GAA AAUUCUAA
1376	GAAACACU C CAAUUUAU	AUAAAUUG CUGAUGA X GAA AGUGUUUC
1381	ACUCCAAU U UAUAGAUU	AAUCUAUA CUGAUGA X GAA AUUGGAGU
1382	CUCCAAUU U AUAGAUUC	GAAUCUAU CUGAUGA X GAA AAUUGGAG
1383	UCCAAUUU A UAGAUUCU	AGAAUCUA CUGAUGA X GAA AAAUUGGA
1385	CAAUUUAU A GAUUCUUU	AAAGAAUC CUGAUGA X GAA AUAAAUUG
1389	UUAUAGAU U CUUUCUUA	UAAGAAAG CUGAUGA X GAA AUCUAUAA
1390	UAUAGAUU C UUUCUUAA	UUAAGAAA CUGAUGA X GAA AAUCUAUA
1392	UAGAUUCU U UCUUAAAC	GUUUAAGA CUGAUGA X GAA AGAAUCUA
1393	AGAUUCUU U CUUAAACA	UGUUUAAG CUGAUGA X GAA AAGAAUCU
1394	GAUUCUUU C UUAAACAC	GUGUUUAA CUGAUGA X GAA AAAGAAUC
1396	UUCUUUCU U AAACACUU	AAGUGUUU CUGAUGA X GAA AGAAAGAA
1397	UCUUUCUU A AACACUUC	GAAGUGUU CUGAUGA X GAA AAGAAAGA

nt. Posi-tion	Target Sequence	Ribozyme Sequence
1404	UAAACACU U CCAGUAAC	GUUACUGG CUGAUGA X GAA AGUGUUUA
1405	AAACACUU C CAGUAACC	GGUUACUG CUGAUGA X GAA AAGUGUUU
1410	CUUCCAGU A ACCAUGAA	UUCAUGGU CUGAUGA X GAA ACUGGAAG
1423	UGAAAACU C AGACUUGG	CCAAGUCU CUGAUGA X GAA AGUUUUCA
1429	CUCAGACU U GGAAAUGC	GCAUUUCC CUGAUGA X GAA AGUCUGAG
1440	AAAUGCCU U CUUUAACU	AGUUAAAG CUGAUGA X GAA AGGCAUUU
1441	AAUGCCUU C UUUAACUU	AAGUUAAA CUGAUGA X GAA AAGGCAUU
1443	UGCCUUCU U UAACUUCC	GGAAGUUA CUGAUGA X GAA AGAAGGCA
1444	GCCUUCUU U AACUUCCA	UGGAAGUU CUGAUGA X GAA AAGAAGGC
1445	CCUUCUUU A ACUUCCAC	GUGGAAGU CUGAUGA X GAA AAAGAAGG
1449	CUUUAACU U CCACCCCC	GGGGGUGG CUGAUGA X GAA AGUUAAAG
1450	UUUAACUU C CACCCCCC	GGGGGGUG CUGAUGA X GAA AAGUUAAA
1460	ACCCCCCU C AUUGGUCA	UGACCAAU CUGAUGA X GAA AGGGGGGU
1463	CCCCUCAU U GGUCACAA	UUGUGACC CUGAUGA X GAA AUGAGGGG
1467	UCAUUGGU C ACAAAUUG	CAAUUUGU CUGAUGA X GAA ACCAAUGA
1474	UCACAAAU U GACUGUUA	UAACAGUC CUGAUGA X GAA AUUUGUGA
1481	UUGACUGU U ACAACACC	GGUGUUGU CUGAUGA X GAA ACAGUCAA
1482	UGACUGUU A CAACACCA	UGGUGUUG CUGAUGA X GAA AACAGUCA
1492	AACACCAU U UCAUAGAG	CUCUAUGA CUGAUGA X GAA AUGGUGUU
1493	ACACCAUU U CAUAGAGA	UCUCUAUG CUGAUGA X GAA AAUGGUGU
1494	CACCAUUU C AUAGAGAC	GUCUCUAU CUGAUGA X GAA AAAUGGUG
1497	CAUUUCAU A GAGACCAG	CUGGUCUC CUGAUGA X GAA AUGAAAUG
1518	UGAAAACU C AAAAGGAA	UUCCUUUU CUGAUGA X GAA AGUUUUCA
1530	AGGAAAAU A CUGUUUUU	AAAAACAG CUGAUGA X GAA AUUUUCCU
1535	AAUACUGU U UUUAGAAC	GUUCUAAA CUGAUGA X GAA ACAGUAUU
1536	AUACUGUU U UUAGAACC	GGUUCUAA CUGAUGA X GAA AACAGUAU
1537	UACUGUUU U UAGAACCC	GGGUUCUA CUGAUGA X GAA AAACAGUA
1538	ACUGUUUU U AGAACCCC	GGGGUUCU CUGAUGA X GAA AAAACAGU
1539	CUGUUUUU A GAACCCCA	UGGGGUUC CUGAUGA X GAA AAAAACAG
1551	CCCCAGCU A UCAAAAGG	CCUUUUGA CUGAUGA X GAA AGCUGGGG
1553	CCAGCUAU C AAAAGGUC	GACCUUUU CUGAUGA X GAA AUAGCUGG
1561	CAAAAGGU C AAUCUUAG	CUAAGAUU CUGAUGA X GAA ACCUUUUG
1565	AGGUCAAU C UUAGAAAG	CUUUCUAA CUGAUGA X GAA AUUGACCU
1567	GUCAAUCU U AGAAAGCU	AGCUUUCU CUGAUGA X GAA AGAUUGAC
1568	UCAAUCUU A GAAAGCUC	GAGCUUUC CUGAUGA X GAA AAGAUUGA
1578	AAAGCUCU C CAAGAACU	AGUUCUUG CUGAUGA X GAA AGAGCUUU

nt. Position	Target Sequence	Ribozyme Sequence
1587	CAAGAACU C CUACACCA	UGGUGUAG CUGAUGA X GAA AGUUCUUG
1590	GAACUCCU A CACCAUUC	GAAUGGUG CUGAUGA X GAA AGGAGUUC
1597	UACACCAU U CAAACAUG	CAUGUUUG CUGAUGA X GAA AUGGUGUA
1598	ACACCAUU C AAACAUGC	GCAUGUUU CUGAUGA X GAA AAUGGUGU
1610	CAUGCACU U GCAGCUCA	UGAGCUGC CUGAUGA X GAA AGUGCAUG
1617	UUGCAGCU C AAGAAAUU	AAUUUCUU CUGAUGA X GAA AGCUGCAA
1625	CAAGAAAU U AAAUACGG	CCGUAUUU CUGAUGA X GAA AUUUCUUG
1626	AAGAAAUU A AAUACGGU	ACCGUAUU CUGAUGA X GAA AAUUUCUU
1635	AAUACGGU C CCCUGAAG	CUUCAGGG CUGAUGA X GAA ACCGUAUU
1649	AAGAUGCU A CCUCAGAC	GUCUGAGG CUGAUGA X GAA AGCAUCUU
1653	UGCUACCU C AGACACCC	GGGUGUCU CUGAUGA X GAA AGGUAGCA
1663	GACACCCU C UCAUCUAG	CUAGAUGA CUGAUGA X GAA AGGGUGUC
1665	CACCCUCU C AUCUAGUA	UACUAGAU CUGAUGA X GAA AGAGGGUG
1668	CCUCUCAU C UAGUAGAA	UUCUACUA CUGAUGA X GAA AUGAGAGG
1670	UCUCAUCU A GUAGAAGA	UCUUCUAC CUGAUGA X GAA AGAUGAGA
1673	CAUCUAGU A GAAGAUCU	AGAUCUUC CUGAUGA X GAA ACUAGAUG
1680	UAGAAGAU C UGCAGGAU	AUCCUGCA CUGAUGA X GAA AUCUUCUA
1694	GAUGUGAU C AAACAGGA	UCCUGUUU CUGAUGA X GAA AUCACAUC
1705	ACAGGAAU C UGAUGAAU	AUUCAUCA CUGAUGA X GAA AUUCCUGU
1714	UGAUGAAU C UGGAAUUG	CAAUUCCA CUGAUGA X GAA AUUCAUCA
1721	UCUGGAAU U GUUGCUGA	UCAGCAAC CUGAUGA X GAA AUUCCAGA
1724	GGAAUUGU U GCUGAGUU	AACUCAGC CUGAUGA X GAA ACAAUUCC
1732	UGCUGAGU U UCAAGAAA	UUUCUUGA CUGAUGA X GAA ACUCAGCA
1733	GCUGAGUU U CAAGAAAA	UUUUCUUG CUGAUGA X GAA AACUCAGC
1753	ACCACCCU U ACUGAAGA	UCUUCAGU CUGAUGA X GAA AGGGUGGU
1754	CCACCCUU A CUGAAGAA	UUCUUCAG CUGAUGA X GAA AAGGGUGG
1766	AAGAAAAU C AAACAAGA	UCUUGUUU CUGAUGA X GAA AUUUUCUU
1783	GGUGGAAU C UCCAACUG	CAGUUGGA CUGAUGA X GAA AUUCCACC
1785	UGGAAUCU C CAACUGAU	AUCAGUUG CUGAUGA X GAA AGAUUCCA
1794	CAACUGAU A AAUCAGGA	UCCUGAUU CUGAUGA X GAA AUCAGUUG
1798	UGAUAAAU C AGGAAACU	AGUUUCCU CUGAUGA X GAA AUUUAUCA
1807	AGGAAACU U CUUCUGCU	AGCAGAAG CUGAUGA X GAA AGUUUCCU
1808	GGAAACUU C UUCUGCUC	GAGCAGAA CUGAUGA X GAA AAGUUUCC
1810	AAACUUCU U CUGCUCAC	GUGAGCAG CUGAUGA X GAA AGAAGUUU
1811	AACUUCUU C UGCUCACA	UGUGAGCA CUGAUGA X GAA AAGAAGUU
1816	CUUCUGCU C ACACCACU	AGUGGUGU CUGAUGA X GAA AGCAGAAG

nt. Position	Target Sequence	Ribozyme Sequence
1839	GGGACAGU C UGAAUACC	GGUAUUCA CUGAUGA X GAA ACUGUCCC
1845	GUCUGAAU A CCCAACUG	CAGUUGGG CUGAUGA X GAA AUUCAGAC
1855	CCAACUGU U CACGCAGA	UCUGCGUG CUGAUGA X GAA ACAGUUGG
1856	CAACUGUU C ACGCAGAC	GUCUGCGU CUGAUGA X GAA AACAGUUG
1867	GCAGACCU C GCCUGUGG	CCACAGGC CUGAUGA X GAA AGGUCUGC
1890	CACCGAAU A UUCUUACA	UGUAAGAA CUGAUGA X GAA AUUCGGUG
1892	CCGAAUAU U CUUACAAG	CUUGUAAG CUGAUGA X GAA AUAUUCGG
1893	CGAAUAUU C UUACAAGC	GCUUGUAA CUGAUGA X GAA AAUAUUCG
1895	AAUAUUCU U ACAAGCUC	GAGCUUGU CUGAUGA X GAA AGAAUAUU
1896	AUAUUCUU A CAAGCUCC	GGAGCUUG CUGAUGA X GAA AAGAAUAU
1903	UACAAGCU C CGUUUUAA	UUAAAACG CUGAUGA X GAA AGCUUGUA
1907	AGCUCCGU U UUAAUGGC	GCCAUUAA CUGAUGA X GAA ACGGAGCU
1908	GCUCCGUU U UAAUGGCA	UGCCAUUA CUGAUGA X GAA AACGGAGC
1909	CUCCGUUU U AAUGGCAC	GUGCCAUU CUGAUGA X GAA AAACGGAG
1910	UCCGUUUU A AUGGCACC	GGUGCCAU CUGAUGA X GAA AAAACGGA
1924	ACCAGCAU C AGAAGAUG	CAUCUUCU CUGAUGA X GAA AUGCUGGU
1943	GACAAUGU U CUCAAAGC	GCUUUGAG CUGAUGA X GAA ACAUUGUC
1944	ACAAUGUU C UCAAAGCA	UGCUUUGA CUGAUGA X GAA AACAUUGU
1946	AAUGUUCU C AAAGCAUU	AAUGCUUU CUGAUGA X GAA AGAACAUU
1954	CAAAGCAU U UACAGUAC	GUACUGUA CUGAUGA X GAA AUGCUUUG
1955	AAAGCAUU U ACAGUACC	GGUACUGU CUGAUGA X GAA AAUGCUUU
1956	AAGCAUUU A CAGUACCU	AGGUACUG CUGAUGA X GAA AAAUGCUU
1961	UUUACAGU A CCUAAAAA	UUUUUAGG CUGAUGA X GAA ACUGUAAA
1965	CAGUACCU A AAAACAGG	CCUGUUUU CUGAUGA X GAA AGGUACUG
1975	AAACAGGU C CCUGGCGA	UCGCCAGG CUGAUGA X GAA ACCUGUUU
1990	GAGCCCCU U GCAGCCUU	AAGGCUGC CUGAUGA X GAA AGGGGCUC
1998	UGCAGCCU U GUAGCAGU	ACUGCUAC CUGAUGA X GAA AGGCUGCA
2001	AGCCUUGU A GCAGUACC	GGUACUGC CUGAUGA X GAA ACAAGGCU
2007	GUAGCAGU A CCUGGGAA	UUCCCAGG CUGAUGA X GAA ACUGCUAC
2023	ACCUGCAU C CUGUGGAA	UUCCACAG CUGAUGA X GAA AUGCAGGU
2053	GAUGACAU C UUCCAGUC	GACUGGAA CUGAUGA X GAA AUGUCAUC
2055	UGACAUCU U CCAGUCAA	UUGACUGG CUGAUGA X GAA AGAUGUCA
2056	GACAUCUU C CAGUCAAG	CUUGACUG CUGAUGA X GAA AAGAUGUC
2061	CUUCCAGU C AAGCUCGU	ACGAGCUU CUGAUGA X GAA ACUGGAAG
2067	GUCAAGCU C GUAAAUAC	GUAUUUAC CUGAUGA X GAA AGCUUGAC
2070	AAGCUCGU A AAUACGUG	CACGUAUU CUGAUGA X GAA ACGAGCUU

nt. Position	Target Sequence	Ribozyme Sequence
2074	UCGUAAAU A CGUGAAUG	CAUUCACG CUGAUGA X GAA AUUUACGA
2086	GAAUGCAU U CUCAGCCC	GGGCUGAG CUGAUGA X GAA AUGCAUUC
2087	AAUGCAUU C UCAGCCCG	CGGGCUGA CUGAUGA X GAA AAUGCAUU
2089	UGCAUUCU C AGCCCGGA	UCCGGGCU CUGAUGA X GAA AGAAUGCA
2105	ACGCUGGU C AUGUGAGA	UCUCACAU CUGAUGA X GAA ACCAGCGU
2117	UGAGACAU U UCCAGAAA	UUUCUGGA CUGAUGA X GAA AUGUCUCA
2118	GAGACAUU U CCAGAAAA	UUUUCUGG CUGAUGA X GAA AAUGUCUC
2119	AGACAUUU C CAGAAAAG	CUUUUCUG CUGAUGA X GAA AAAUGUCU
2131	AAAAGCAU U AUGGUUUU	AAAACCAU CUGAUGA X GAA AUGCUUUU
2132	AAAGCAUU A UGGUUUUC	GAAAACCA CUGAUGA X GAA AAUGCUUU
2137	AUUAUGGU U UUCAGAAC	GUUCUGAA CUGAUGA X GAA ACCAUAAU
2138	UUAUGGUU U UCAGAACA	UGUUCUGA CUGAUGA X GAA AACCAUAA
2139	UAUGGUUU U CAGAACAC	GUGUUCUG CUGAUGA X GAA AAACCAUA
2140	AUGGUUUU C AGAACACU	AGUGUUCU CUGAUGA X GAA AAAACCAU
2149	AGAACACU U CAAGUUGA	UCAACUUG CUGAUGA X GAA AGUGUUCU
2150	GAACACUU C AAGUUGAC	GUCAACUU CUGAUGA X GAA AAGUGUUC
2155	CUUCAAGU U GACUUGGG	CCCAAGUC CUGAUGA X GAA ACUUGAAG
2160	AGUUGACU U GGGAUAUA	UAUAUCCC CUGAUGA X GAA AGUCAACU
2166	CUUGGGAU A UAUCAUUC	GAAUGAUA CUGAUGA X GAA AUCCCAAG
2168	UGGGAUAU A UCAUUCCU	AGGAAUGA CUGAUGA X GAA AUAUCCCA
2170	GGAUAUAU C AUUCCUCA	UGAGGAAU CUGAUGA X GAA AUAUAUCC
2173	UAUAUCAU U CCUCAACA	UGUUGAGG CUGAUGA X GAA AUGAUAUA
2174	AUAUCAUU C CUCAACAU	AUGUUGAG CUGAUGA X GAA AAUGAUAU
2177	UCAUUCCU C AACAUGAA	UUCAUGUU CUGAUGA X GAA AGGAAUGA
2189	AUGAAACU U UUCAUGAA	UUCAUGAA CUGAUGA X GAA AGUUUCAU
2190	UGAAACUU U UCAUGAAU	AUUCAUGA CUGAUGA X GAA AAGUUUCA
2191	GAAACUUU U CAUGAAUG	CAUUCAUG CUGAUGA X GAA AAAGUUUC
2192	AAACUUUU C AUGAAUGG	CCAUUCAU CUGAUGA X GAA AAAAGUUU
2212	AAGAACCU A UUUUUGUU	AACAAAAA CUGAUGA X GAA AGGUUCUU
2214	GAACCUAU U UUUGUUGU	ACAACAAA CUGAUGA X GAA AUAGGUUC
2215	AACCUAUU U UUGUUGUG	CACAACAA CUGAUGA X GAA AAUAGGUU
2216	ACCUAUUU U UGUUGUGG	CCACAACA CUGAUGA X GAA AAAUAGGU
2217	CCUAUUUU U GUUGUGGU	ACCACAAC CUGAUGA X GAA AAAAUAGG
2220	AUUUUUGU U GUGGUACA	UGUACCAC CUGAUGA X GAA ACAAAAAU
2226	GUUGUGGU A CAACAGUU	AACUGUUG CUGAUGA X GAA ACCACAAC
2234	ACAACAGU U GAGAGCAG	CUGCUCUC CUGAUGA X GAA ACUGUUGU

nt. Posi-tion	Target Sequence	Ribozyme Sequence
2255	AAGUGCAU U UAGUUGAA	UUCAACUA CUGAUGA X GAA AUGCACUU
2256	AGUGCAUU U AGUUGAAU	AUUCAACU CUGAUGA X GAA AAUGCACU
2257	GUGCAUUU A GUUGAAUG	CAUUCAAC CUGAUGA X GAA AAAUGCAC
2260	CAUUUAGU U GAAUGAAG	CUUCAUUC CUGAUGA X GAA ACUAAAUG
2270	AAUGAAGU C UUCUUGGA	UCCAAGAA CUGAUGA X GAA ACUUCAUU
2272	UGAAGUCU U CUUGGAUU	AAUCCAAG CUGAUGA X GAA AGACUUCA
2273	GAAGUCUU C UUGGAUUU	AAAUCCAA CUGAUGA X GAA AAGACUUC
2275	AGUCUUCU U GGAUUUCA	UGAAAUCC CUGAUGA X GAA AGAAGACU
2280	UCUUGGAU U UCACCCAA	UUGGGUGA CUGAUGA X GAA AUCCAAGA
2281	CUUGGAUU U CACCCAAC	GUUGGGUG CUGAUGA X GAA AAUCCAAG
2282	UUGGAUUU C ACCCAACU	AGUUGGGU CUGAUGA X GAA AAAUCCAA
2291	ACCCAACU A AAAGGAUU	AAUCCUUU CUGAUGA X GAA AGUUGGGU
2299	AAAAGGAU U UUUAAAAA	UUUUUAAA CUGAUGA X GAA AUCCUUUU
2300	AAAGGAUU U UUAAAAAU	AUUUUUAA CUGAUGA X GAA AAUCCUUU
2301	AAGGAUUU U UAAAAAUA	UAUUUUUA CUGAUGA X GAA AAAUCCUU
2302	AGGAUUUU U AAAAAUAA	UUAUUUUU CUGAUGA X GAA AAAAUCCU
2309	UUAAAAAU A AAUAACAG	CUGUUAUU CUGAUGA X GAA AUUUUUAA
2313	AAAUAAAU A ACAGUCUU	AAGACUGU CUGAUGA X GAA AUUUAUUU
2319	AUAACAGU C UUACCUAA	UUAGGUAA CUGAUGA X GAA ACUGUUAU
2321	AACAGUCU U ACCUAAAU	AUUUAGGU CUGAUGA X GAA AGACUGUU
2322	ACAGUCUU A CCUAAAUU	AAUUUAGG CUGAUGA X GAA AAGACUGU
2326	UCUUACCU A AAUUAUUA	UAAUAAUU CUGAUGA X GAA AGGUAAGA
2330	ACCUAAAU U AUUAGGUA	UACCUAAU CUGAUGA X GAA AUUUAGGU
2331	CCUAAAUU A UUAGGUAA	UUACCUAA CUGAUGA X GAA AAUUUAGG
2333	UAAAUUAU U AGGUAAUG	CAUUACCU CUGAUGA X GAA AUAAUUUA
2334	AAAUUAUU A GGUAAUGA	UCAUUACC CUGAUGA X GAA AAUAAUUU
2338	UAUUAGGU A AUGAAUUG	CAAUUCAU CUGAUGA X GAA ACCUAAUA
2345	UAAUGAAU U GUAGCCAG	CUGGCUAC CUGAUGA X GAA AUUCAUUA
2348	UGAAUUGU A GCCAGUUG	CAACUGGC CUGAUGA X GAA ACAAUUCA
2355	UAGCCAGU U GUUAAUAU	AUAUUAAC CUGAUGA X GAA ACUGGCUA
2358	CCAGUUGU U AAUAUCUU	AAGAUAUU CUGAUGA X GAA ACAACUGG
2359	CAGUUGUU A AUAUCUUA	UAAGAUAU CUGAUGA X GAA AACAACUG
2362	UUGUUAAU A UCUUAAUG	CAUUAAGA CUGAUGA X GAA AUUAACAA
2364	GUUAAUAU C UUAAUGCA	UGCAUUAA CUGAUGA X GAA AUAUUAAC
2366	UAAUAUCU U AAUGCAGA	UCUGCAUU CUGAUGA X GAA AGAUAUUA
2367	AAUAUCUU A AUGCAGAU	AUCUGCAU CUGAUGA X GAA AAGAUAUU

nt. Position	Target Sequence	Ribozyme Sequence
2376	AUGCAGAU U UUUUUAAA	UUUAAAAA CUGAUGA X GAA AUCUGCAU
2377	UGCAGAUU U UUUUAAAA	UUUUAAAA CUGAUGA X GAA AAUCUGCA
2378	GCAGAUUU U UUUAAAAA	UUUUUAAA CUGAUGA X GAA AAAUCUGC
2379	CAGAUUUU U UUAAAAAA	UUUUUUAA CUGAUGA X GAA AAAAUCUG
2380	AGAUUUUU U UAAAAAAA	UUUUUUUA CUGAUGA X GAA AAAAAUCU
2381	GAUUUUUU U AAAAAAAA	UUUUUUUU CUGAUGA X GAA AAAAAAUC
2382	AUUUUUUU A AAAAAAAC	GUUUUUUU CUGAUGA X GAA AAAAAAAU
2393	AAAAACAU A AAAUGAUU	AAUCAUUU CUGAUGA X GAA AUGUUUUU
2401	AAAAUGAU U UAUCUGUA	UACAGAUA CUGAUGA X GAA AUCAUUUU
2402	AAAUGAUU U AUCUGUAU	AUACAGAU CUGAUGA X GAA AAUCAUUU
2403	AAUGAUUU A UCUGUAUU	AAUACAGA CUGAUGA X GAA AAAUCAUU
2405	UGAUUUAU C UGUAUUUU	AAAAUACA CUGAUGA X GAA AUAAAUCA
2409	UUAUCUGU A UUUUAAAG	CUUUAAAA CUGAUGA X GAA ACAGAUAA
2411	AUCUGUAU U UUAAAGGA	UCCUUUAA CUGAUGA X GAA AUACAGAU
2412	UCUGUAUU U UAAAGGAU	AUCCUUUA CUGAUGA X GAA AAUACAGA
2413	CUGUAUUU U AAAGGAUC	GAUCCUUU CUGAUGA X GAA AAAUACAG
2414	UGUAUUUU A AAGGAUCC	GGAUCCUU CUGAUGA X GAA AAAAUACA
2421	UAAAGGAU C CAACAGAU	AUCUGUUG CUGAUGA X GAA AUCCUUUA
2430	CAACAGAU C AGUAUUUU	AAAAUACU CUGAUGA X GAA AUCUGUUG
2434	AGAUCAGU A UUUUUUCC	GGAAAAAA CUGAUGA X GAA ACUGAUCU
2436	AUCAGUAU U UUUUCCUG	CAGGAAAA CUGAUGA X GAA AUACUGAU
2437	UCAGUAUU U UUUCCUGU	ACAGGAAA CUGAUGA X GAA AAUACUGA
2438	CAGUAUUU U UUCCUGUG	CACAGGAA CUGAUGA X GAA AAAUACUG
2439	AGUAUUUU U UCCUGUGA	UCACAGGA CUGAUGA X GAA AAAAUACU
2440	GUAUUUUU U CCUGUGAU	AUCACAGG CUGAUGA X GAA AAAAAUAC
2441	UAUUUUUU C CUGUGAUG	CAUCACAG CUGAUGA X GAA AAAAAAUA
2453	UGAUGGGU U UUUUGAAA	UUUCAAAA CUGAUGA X GAA ACCCAUCA
2454	GAUGGGUU U UUUGAAAU	AUUUCAAA CUGAUGA X GAA AACCCAUC
2455	AUGGGUUU U UUGAAAUU	AAUUUCAA CUGAUGA X GAA AAACCCAU
2456	UGGGUUUU U UGAAAUUU	AAAUUUCA CUGAUGA X GAA AAAACCCA
2457	GGGUUUUU U GAAAUUUG	CAAAUUUC CUGAUGA X GAA AAAAACCC
2463	UUUGAAAU U UGACACAU	AUGUGUCA CUGAUGA X GAA AUUUCAAA
2464	UUGAAAUU U GACACAUU	AAUGUGUC CUGAUGA X GAA AAUUUCAA
2472	UGACACAU U AAAAGGUA	UACCUUUU CUGAUGA X GAA AUGUGUCA
2473	GACACAUU A AAAGGUAC	GUACCUUU CUGAUGA X GAA AAUGUGUC
2480	UAAAAGGU A CUCCAGUA	UACUGGAG CUGAUGA X GAA ACCUUUUA

nt. Position	Target Sequence	Ribozyme Sequence
2488	ACUCCAGU A UUUCACUU	AAGUGAAA CUGAUGA X GAA ACUGGAGU
2490	UCCAGUAU U UCACUUUU	AAAAGUGA CUGAUGA X GAA AUACUGGA
2491	CCAGUAUU U CACUUUUC	GAAAAGUG CUGAUGA X GAA AAUACUGG
2492	CAGUAUUU C ACUUUUCU	AGAAAAGU CUGAUGA X GAA AAAUACUG
2496	AUUUCACU U UUCUCGAU	AUCGAGAA CUGAUGA X GAA AGUGAAAU
2497	UUUCACUU U UCUCGAUC	GAUCGAGA CUGAUGA X GAA AAGUGAAA
2498	UUCACUUU U CUCGAUCA	UGAUCGAG CUGAUGA X GAA AAAGUGAA
2501	ACUUUUCU C GAUCACUA	UAGUGAUC CUGAUGA X GAA AGAAAAGU
2505	UUCUCGAU C ACUAAACA	UGUUUAGU CUGAUGA X GAA AUCGAGAA
2509	CGAUCACU A AACAUAUG	CAUAUGUU CUGAUGA X GAA AGUGAUCG
2515	CUAAACAU A UGCAUAUA	UAUAUGCA CUGAUGA X GAA AUGUUUAG
2521	AUAUGCAU A UAUUUUUA	UAAAAAUA CUGAUGA X GAA AUGCAUAU
2523	AUGCAUAU A UUUUUAAA	UUUAAAAA CUGAUGA X GAA AUAUGCAU
2525	GCAUAUAU U UUUAAAAA	UUUUUAAA CUGAUGA X GAA AUAUAUGC
2526	CAUAUAUU U UUAAAAAU	AUUUUUAA CUGAUGA X GAA AAUAUAUG
2527	AUAUAUUU U UAAAAAUC	GAUUUUUA CUGAUGA X GAA AAAUAUAU
2528	UAUAUUUU U AAAAAUCA	UGAUUUUU CUGAUGA X GAA AAAAUAUA
2529	AUAUUUUU A AAAAUCAG	CUGAUUUU CUGAUGA X GAA AAAAAUAU
2535	UUAAAAAU C AGUAAAAG	CUUUUACU CUGAUGA X GAA AUUUUUAA
2539	AAAUCAGU A AAAGCAUU	AAUGCUUU CUGAUGA X GAA ACUGAUUU
2547	AAAAGCAU U ACUCUAAG	CUUAGAGU CUGAUGA X GAA AUGCUUUU
2548	AAAGCAUU A CUCUAAGU	ACUUAGAG CUGAUGA X GAA AAUGCUUU
2551	GCAUUACU C UAAGUGUA	UACACUUA CUGAUGA X GAA AGUAAUGC
2553	AUUACUCU A AGUGUAGA	UCUACACU CUGAUGA X GAA AGAGUAAU
2559	CUAAGUGU A GACUUAAU	AUUAAGUC CUGAUGA X GAA ACACUUAG
2564	UGUAGACU U AAUACCAU	AUGGUAUU CUGAUGA X GAA AGUCUACA
2565	GUAGACUU A AUACCAUG	CAUGGUAU CUGAUGA X GAA AAGUCUAC
2568	GACUUAAU A CCAUGUGA	UCACAUGG CUGAUGA X GAA AUUAAGUC
2580	UGUGACAU U UAAUCCAG	CUGGAUUA CUGAUGA X GAA AUGUCACA
2581	GUGACAUU U AAUCCAGA	UCUGGAUU CUGAUGA X GAA AAUGUCAC
2582	UGACAUUU A AUCCAGAU	AUCUGGAU CUGAUGA X GAA AAAUGUCA
2585	CAUUUAAU C CAGAUUGU	ACAAUCUG CUGAUGA X GAA AUUAAAUG
2591	AUCCAGAU U GUAAAUGC	GCAUUUAC CUGAUGA X GAA AUCUGGAU
2594	CAGAUUGU A AAUGCUCA	UGAGCAUU CUGAUGA X GAA ACAAUCUG
2601	UAAAUGCU C AUUUAUGG	CCAUAAAU CUGAUGA X GAA AGCAUUUA
2604	AUGCUCAU U UAUGGUUA	UAACCAUA CUGAUGA X GAA AUGAGCAU

nt. Position	Target Sequence	Ribozyme Sequence
2605	UGCUCAUU U AUGGUUAA	UUAACCAU CUGAUGA X GAA AAUGAGCA
2606	GCUCAUUU A UGGUUAAU	AUUAACCA CUGAUGA X GAA AAAUGAGC
2611	UUUAUGGU U AAUGACAU	AUGUCAUU CUGAUGA X GAA ACCAUAAA
2612	UUAUGGUU A AUGACAUU	AAUGUCAU CUGAUGA X GAA AACCAUAA
2620	AAUGACAU U GAAGGUAC	GUACCUUC CUGAUGA X GAA AUGUCAUU
2627	UUGAAGGU A CAUUUAUU	AAUAAAUG CUGAUGA X GAA ACCUUCAA
2631	AGGUACAU U UAUUGUAC	GUACAAUA CUGAUGA X GAA AUGUACCU
2632	GGUACAUU U AUUGUACC	GGUACAAU CUGAUGA X GAA AAUGUACC
2633	GUACAUUU A UUGUACCA	UGGUACAA CUGAUGA X GAA AAAUGUAC
2635	ACAUUUAU U GUACCAAA	UUUGGUAC CUGAUGA X GAA AUAAAUGU
2638	UUUAUUGU A CCAAACCA	UGGUUUGG CUGAUGA X GAA ACAAUAAA
2648	CAAACCAU U UUAUGAGU	ACUCAUAA CUGAUGA X GAA AUGGUUUG
2649	AAACCAUU U UAUGAGUU	AACUCAUA CUGAUGA X GAA AAUGGUUU
2650	AACCAUUU U AUGAGUUU	AAACUCAU CUGAUGA X GAA AAAUGGUU
2651	ACCAUUUU A UGAGUUUU	AAAACUCA CUGAUGA X GAA AAAAUGGU
2657	UUAUGAGU U UUCUGUUA	UAACAGAA CUGAUGA X GAA ACUCAUAA
2658	UAUGAGUU U UCUGUUAG	CUAACAGA CUGAUGA X GAA AACUCAUA
2659	AUGAGUUU U CUGUUAGC	GCUAACAG CUGAUGA X GAA AAACUCAU
2660	UGAGUUUU C UGUUAGCU	AGCUAACA CUGAUGA X GAA AAAACUCA
2664	UUUUCUGU U AGCUUGCU	AGCAAGCU CUGAUGA X GAA ACAGAAAA
2665	UUUCUGUU A GCUUGCUU	AAGCAAGC CUGAUGA X GAA AACAGAAA
2669	UGUUAGCU U GCUUUAAA	UUUAAAGC CUGAUGA X GAA AGCUAACA
2673	AGCUUGCU U UAAAAAUU	AAUUUUUA CUGAUGA X GAA AGCAAGCU
2674	GCUUGCUU U AAAAAUUA	UAAUUUUU CUGAUGA X GAA AAGCAAGC
2675	CUUGCUUU A AAAAUUAU	AUAAUUUU CUGAUGA X GAA AAAGCAAG
2681	UUAAAAAU U AUUACUGU	ACAGUAAU CUGAUGA X GAA AUUUUUAA
2682	UAAAAAUU A UUACUGUA	UACAGUAA CUGAUGA X GAA AAUUUUUA
2684	AAAAUUAU U ACUGUAAG	CUUACAGU CUGAUGA X GAA AUAAUUUU
2685	AAAUUAUU A CUGUAAGA	UCUUACAG CUGAUGA X GAA AAUAAUUU
2690	AUUACUGU A AGAAAUAG	CUAUUUCU CUGAUGA X GAA ACAGUAAU
2697	UAAGAAAU A GUUUUAUA	UAUAAAAC CUGAUGA X GAA AUUUCUUA
2700	GAAAUAGU U UUAUAAAA	UUUUAUAA CUGAUGA X GAA ACUAUUUC
2701	AAAUAGUU U UAUAAAAA	UUUUUAUA CUGAUGA X GAA AACUAUUU
2702	AAUAGUUU U AUAAAAAA	UUUUUUAU CUGAUGA X GAA AAACUAUU
2703	AUAGUUUU A UAAAAAAU	AUUUUUUA CUGAUGA X GAA AAAACUAU
2705	AGUUUUAU A AAAAAUUA	UAAUUUUU CUGAUGA X GAA AUAAAACU

nt. Position	Target Sequence	Ribozyme Sequence
2712	UAAAAAAU U AUAUUUUU	AAAAAUAU CUGAUGA X GAA AUUUUUUA
2713	AAAAAAUU A UAUUUUUA	UAAAAAUA CUGAUGA X GAA AAUUUUUU
2715	AAAAUUAU A UUUUUAUU	AAUAAAAA CUGAUGA X GAA AUAAUUUU
2717	AAUUAUAU U UUUAUUCA	UGAAUAAA CUGAUGA X GAA AUAUAAUU
2718	AUUAUAUU U UUAUUCAG	CUGAAUAA CUGAUGA X GAA AAUAUAAU
2719	UUAUAUUU U UAUUCAGU	ACUGAAUA CUGAUGA X GAA AAAUAUAA
2720	UAUAUUUU U AUUCAGUA	UACUGAAU CUGAUGA X GAA AAAAUAUA
2721	AUAUUUUU A UUCAGUAA	UUACUGAA CUGAUGA X GAA AAAAAUAU
2723	AUUUUUAU U CAGUAAUU	AAUUACUG CUGAUGA X GAA AUAAAAAU
2724	UUUUUAUU C AGUAAUUU	AAAUUACU CUGAUGA X GAA AAUAAAAA
2728	UAUUCAGU A AUUUAAUU	AAUUAAAU CUGAUGA X GAA ACUGAAUA
2731	UCAGUAAU U UAAUUUUG	CAAAAUUA CUGAUGA X GAA AUUACUGA
2732	CAGUAAUU U AAUUUUGU	ACAAAAUU CUGAUGA X GAA AAUUACUG
2733	AGUAAUUU A AUUUUGUA	UACAAAAU CUGAUGA X GAA AAAUUACU
2736	AAUUUAAU U UUGUAAAU	AUUUACAA CUGAUGA X GAA AUUAAAUU
2737	AUUUAAUU U UGUAAAUG	CAUUUACA CUGAUGA X GAA AAUUAAAU
2738	UUUAAUUU U GUAAAUGC	GCAUUUAC CUGAUGA X GAA AAAUUAAA
2741	AAUUUUGU A AAUGCCAA	UUGGCAUU CUGAUGA X GAA ACAAAAUU
2761	AAAAACGU U UUUUGCUG	CAGCAAAA CUGAUGA X GAA ACGUUUUU
2762	AAAACGUU U UUUGCUGC	GCAGCAAA CUGAUGA X GAA AACGUUUU
2763	AAACGUUU U UUGCUGCU	AGCAGCAA CUGAUGA X GAA AAACGUUU
2764	AACGUUUU U UGCUGCUA	UAGCAGCA CUGAUGA X GAA AAAACGUU
2765	ACGUUUUU U GCUGCUAU	AUAGCAGC CUGAUGA X GAA AAAAACGU
2772	UUGCUGCU A UGGUCUUA	UAAGACCA CUGAUGA X GAA AGCAGCAA
2777	GCUAUGGU C UUAGCCUG	CAGGCUAA CUGAUGA X GAA ACCAUAGC
2779	UAUGGUCU U AGCCUGUA	UACAGGCU CUGAUGA X GAA AGACCAUA
2780	AUGGUCUU A GCCUGUAG	CUACAGGC CUGAUGA X GAA AAGACCAU
2787	UAGCCUGU A GACAUGCU	AGCAUGUC CUGAUGA X GAA ACAGGCUA
2802	CUGCUAGU A UCAGAGGG	CCCUCUGA CUGAUGA X GAA ACUAGCAG
2804	GCUAGUAU C AGAGGGGC	GCCCCUCU CUGAUGA X GAA AUACUAGC
2816	GGGGCAGU A GAGCUUGG	CCAAGCUC CUGAUGA X GAA ACUGCCCC
2822	GUAGAGCU U GGACAGAA	UUCUGUCC CUGAUGA X GAA AGCUCUAC
2843	AAGAAACU U GGUGUUAG	CUAACACC CUGAUGA X GAA AGUUUCUU
2849	CUUGGUGU U AGGUAAUU	AAUUACCU CUGAUGA X GAA ACACCAAG
2850	UUGGUGUU A GGUAAUUG	CAAUUACC CUGAUGA X GAA AACACCAA
2854	UGUUAGGU A AUUGACUA	UAGUCAAU CUGAUGA X GAA ACCUAACA

nt. Position	Target Sequence	Ribozyme Sequence
2857	UAGGUAAU U GACUAUGC	GCAUAGUC CUGAUGA X GAA AUUACCUA
2862	AAUUGACU A UGCACUAG	CUAGUGCA CUGAUGA X GAA AGUCAAUU
2869	UAUGCACU A GUAUUUCA	UGAAAUAC CUGAUGA X GAA AGUGCAUA
2872	GCACUAGU A UUUCAGAC	GUCUGAAA CUGAUGA X GAA ACUAGUGC
2874	ACUAGUAU U UCAGACUU	AAGUCUGA CUGAUGA X GAA AUACUAGU
2875	CUAGUAUU U CAGACUUU	AAAGUCUG CUGAUGA X GAA AAUACUAG
2876	UAGUAUUU C AGACUUUU	AAAAGUCU CUGAUGA X GAA AAAUACUA
2882	UUCAGACU U UUUAAUUU	AAAUUAAA CUGAUGA X GAA AGUCUGAA
2883	UCAGACUU U UUAAUUUU	AAAAUUAA CUGAUGA X GAA AAGUCUGA
2884	CAGACUUU U UAAUUUUA	UAAAAUUA CUGAUGA X GAA AAAGUCUG
2885	AGACUUUU U AAUUUUAU	AUAAAAUU CUGAUGA X GAA AAAAGUCU
2886	GACUUUUU A AUUUUAUA	UAUAAAAU CUGAUGA X GAA AAAAAGUC
2889	UUUUUAAU U UUAUAUAU	AUAUAUAA CUGAUGA X GAA AUUAAAAA
2890	UUUUAAUU U UAUAUAUA	UAUAUAUA CUGAUGA X GAA AAUUAAAA
2891	UUUAAUUU U AUAUAUAU	AUAUAUAU CUGAUGA X GAA AAAUUAAA
2892	UUAAUUUU A UAUAUAUA	UAUAUAUA CUGAUGA X GAA AAAAUUAA
2894	AAUUUUAU A UAUAUAUA	UAUAUAUA CUGAUGA X GAA AUAAAAUU
2896	UUUUAUAU A UAUAUACA	UGUAUAUA CUGAUGA X GAA AUAUAAAA
2898	UUAUAUAU A UAUACAUU	AAUGUAUA CUGAUGA X GAA AUAUAUAA
2900	AUAUAUAU A UACAUUUU	AAAAUGUA CUGAUGA X GAA AUAUAUAU
2902	AUAUAUAU A CAUUUUUU	AAAAAAUG CUGAUGA X GAA AUAUAUAU
2906	AUAUACAU U UUUUUUCC	GGAAAAAA CUGAUGA X GAA AUGUAUAU
2907	UAUACAUU U UUUUUCCU	AGGAAAAA CUGAUGA X GAA AAUGUAUA
2908	AUACAUUU U UUUUCCUU	AAGGAAAA CUGAUGA X GAA AAAUGUAU
2909	UACAUUUU U UUUCCUUC	GAAGGAAA CUGAUGA X GAA AAAAUGUA
2910	ACAUUUUU U UUCCUUCU	AGAAGGAA CUGAUGA X GAA AAAAAUGU
2911	CAUUUUUU U UCCUUCUG	CAGAAGGA CUGAUGA X GAA AAAAAAUG
2912	AUUUUUUU U CCUUCUGC	GCAGAAGG CUGAUGA X GAA AAAAAAAU
2913	UUUUUUUU C CUUCUGCA	UGCAGAAG CUGAUGA X GAA AAAAAAAA
2916	UUUUUCCU U CUGCAAUA	UAUUGCAG CUGAUGA X GAA AGGAAAAA
2917	UUUUCCUU C UGCAAUAC	GUAUUGCA CUGAUGA X GAA AAGGAAAA
2924	UCUGCAAU A CAUUUGAA	UUCAAAUG CUGAUGA X GAA AUUGCAGA
2928	CAAUACAU U UGAAAACU	AGUUUUCA CUGAUGA X GAA AUGUAUUG
2929	AAUACAUU U GAAAACUU	AAGUUUUC CUGAUGA X GAA AAUGUAUU
2937	UGAAAACU U GUUUGGGA	UCCCAAAC CUGAUGA X GAA AGUUUUCA
2940	AAACUUGU U UGGGAGAC	GUCUCCCA CUGAUGA X GAA ACAAGUUU

nt. Position	Target Sequence	Ribozyme Sequence
2941	AACUUGUU U GGGAGACU	AGUCUCCC CUGAUGA X GAA AACAAGUU
2950	GGGAGACU C UGCAUUUU	AAAAUGCA CUGAUGA X GAA AGUCUCCC
2956	CUCUGCAU U UUUUAUUG	CAAUAAAA CUGAUGA X GAA AUGCAGAG
2957	UCUGCAUU U UUUAUUGU	ACAAUAAA CUGAUGA X GAA AAUGCAGA
2958	CUGCAUUU U UUAUUGUG	CACAAUAA CUGAUGA X GAA AAAUGCAG
2959	UGCAUUUU U UAUUGUGG	CCACAAUA CUGAUGA X GAA AAAAUGCA
2960	GCAUUUUU U AUUGUGGU	ACCACAAU CUGAUGA X GAA AAAAAUGC
2961	CAUUUUUU A UUGUGGUU	AACCACAA CUGAUGA X GAA AAAAAAUG
2969	AUUGUGGU U UUUUUGUU	AACAAAAA CUGAUGA X GAA ACCACAAU
2970	UUGUGGUU U UUUUGUUA	UAACAAAA CUGAUGA X GAA AACCACAA
2971	UGUGGUUU U UUUGUUAU	AUAACAAA CUGAUGA X GAA AAACCACA
2972	GUGGUUUU U UUGUUAUU	AAUAACAA CUGAUGA X GAA AAAACCAC
2973	UGGUUUUU U UGUUAUUG	CAAUAACA CUGAUGA X GAA AAAAACCA
2974	GGUUUUUU U GUUAUUGU	ACAAUAAC CUGAUGA X GAA AAAAAACC
2977	UUUUUUGU U AUUGUUGG	CCAACAAU CUGAUGA X GAA ACAAAAAA
2978	UUUUUGUU A UUGUUGGU	ACCAACAA CUGAUGA X GAA AACAAAAA
2980	UUUGUUAU U GUUGGUUU	AAACCAAC CUGAUGA X GAA AUAACAAA
2983	GUUAUUGU U GGUUUAUA	UAUAAACC CUGAUGA X GAA ACAAUAAC
2987	UUGUUGGU U UAUACAAG	CUUGUAUA CUGAUGA X GAA ACCAACAA
2988	UGUUGGUU U AUACAAGC	GCUUGUAU CUGAUGA X GAA AACCAACA
2989	GUUGGUUU A UACAAGCA	UGCUUGUA CUGAUGA X GAA AAACCAAC
2991	UGGUUUAU A CAAGCAUG	CAUGCUUG CUGAUGA X GAA AUAAACCA
3003	GCAUGCGU U GCACUUCU	AGAAGUGC CUGAUGA X GAA ACGCAUGC
3009	GUUGCACU U CUUUUUUG	CAAAAAAG CUGAUGA X GAA AGUGCAAC
3010	UUGCACUU C UUUUUUGG	CCAAAAAA CUGAUGA X GAA AAGUGCAA
3012	GCACUUCU U UUUUGGGA	UCCCAAAA CUGAUGA X GAA AGAAGUGC
3013	CACUUCUU U UUUGGGAG	CUCCCAAA CUGAUGA X GAA AAGAAGUG
3014	ACUUCUUU U UUGGGAGA	UCUCCCAA CUGAUGA X GAA AAAGAAGU
3015	CUUCUUUU U UGGGAGAU	AUCUCCCA CUGAUGA X GAA AAAAGAAG
3016	UUCUUUUU U GGGAGAUG	CAUCUCCC CUGAUGA X GAA AAAAAGAA
3030	AUGUGUGU U GUUGAUGU	ACAUCAAC CUGAUGA X GAA ACACACAU
3033	UGUGUUGU U GAUGUUCU	AGAACAUC CUGAUGA X GAA ACAACACA
3039	GUUGAUGU U CUAUGUUU	AAACAUAG CUGAUGA X GAA ACAUCAAC
3042	GAUGUUCU A UGUUUUGU	ACAAAACA CUGAUGA X GAA AGAACAUC
3046	UUCUAUGU U UUGUUUUG	CAAAACAA CUGAUGA X GAA ACAUAGAA
3047	UCUAUGUU U UGUUUUGA	UCAAAACA CUGAUGA X GAA AACAUAGA

nt. Position	Target Sequence			Ribozyme Sequence
3048	CUAUGUUU	U	GUUUUGAG	CUCAAAAC CUGAUGA X GAA AAACAUAG
3051	UGUUUUGU	U	UUGAGUGU	ACACUCAA CUGAUGA X GAA ACAAAACA
3052	GUUUUGUU	U	UGAGUGUA	UACACUCA CUGAUGA X GAA AACAAAAC
3053	UUUUGUUU	U	GAGUGUAG	CUACACUC CUGAUGA X GAA AAACAAAA
3060	UUGAGUGU	A	GCCUGACU	AGUCAGGC CUGAUGA X GAA ACACUCAA
3071	CUGACUGU	U	UUAUAAUU	AAUUAUAA CUGAUGA X GAA ACAGUCAG
3072	UGACUGUU	U	UAUAAUUU	AAAUUAUA CUGAUGA X GAA AACAGUCA
3073	GACUGUUU	U	AUAAUUUG	CAAAUUAU CUGAUGA X GAA AAACAGUC
3074	ACUGUUUU	A	UAAUUUGG	CCAAAUUA CUGAUGA X GAA AAAACAGU
3076	UGUUUUAU	A	AUUUGGGA	UCCCAAAU CUGAUGA X GAA AUAAAACA
3079	UUUAUAAU	U	UGGGAGUU	AACUCCCA CUGAUGA X GAA AUUAUAAA
3080	UUAUAAUU	U	GGGAGUUC	GAACUCCC CUGAUGA X GAA AAUUAUAA
3087	UUGGGAGU	U	CUGCAUUU	AAAUGCAG CUGAUGA X GAA ACUCCCAA
3094	UUCUGCAU	U	UGAUCCGC	GCGGAUCA CUGAUGA X GAA AUGCAGAA
3095	UCUGCAUU	U	GAUCCGCA	UGCGGAUC CUGAUGA X GAA AAUGCAGA
3099	CAUUUGAU	C	CGCAUCCC	GGGAUGCG CUGAUGA X GAA AUCAAAUG
3105	AUCCGCAU	C	CCCUGUGG	CCACAGGG CUGAUGA X GAA AUGCGGAU
3115	CCUGUGGU	U	UCUAAGUG	CACUUAGA CUGAUGA X GAA ACCACAGG
3116	CUGUGGUU	U	CUAAGUGU	ACACUUAG CUGAUGA X GAA AACCACAG
3117	UGUGGUUU	C	UAAGUGUA	UACACUUA CUGAUGA X GAA AAACCACA
3119	UGGUUUCU	A	AGUGUAUG	CAUACACU CUGAUGA X GAA AGAAACCA
3125	CUAAGUGU	A	UGGUCUCA	UGAGACCA CUGAUGA X GAA ACACUUAG
3130	UGUAUGGU	C	UCAGAACU	AGUUCUGA CUGAUGA X GAA ACCAUACA
3132	UAUGGUCU	C	AGAACUGU	ACAGUUCU CUGAUGA X GAA AGACCAUA
3141	AGAACUGU	U	GCAUGGAU	AUCCAUGC CUGAUGA X GAA ACAGUUCU
3150	GCAUGGAU	C	CUGUGUUU	AAACACAG CUGAUGA X GAA AUCCAUGC
3157	UCCUGUGU	U	UGCAACUG	CAGUUGCA CUGAUGA X GAA ACACAGGA
3158	CCUGUGUU	U	GCAACUGG	CCAGUUGC CUGAUGA X GAA AACACAGG
3185	ACUGUGGU	U	GAUAGCCA	UGGCUAUC CUGAUGA X GAA ACCACAGU
3189	UGGUUGAU	A	GCCAGUCA	UGACUGGC CUGAUGA X GAA AUCAACCA
3196	UAGCCAGU	C	ACUGCCUU	AAGGCAGU CUGAUGA X GAA ACUGGCUA
3204	CACUGCCU	U	AAGAACAU	AUGUUCUU CUGAUGA X GAA AGGCAGUG
3205	ACUGCCUU	A	AGAACAUU	AAUGUUCU CUGAUGA X GAA AAGGCAGU
3213	AAGAACAU	U	UGAUGCAA	UUGCAUCA CUGAUGA X GAA AUGUUCUU
3214	AGAACAUU	U	GAUGCAAG	CUUGCAUC CUGAUGA X GAA AAUGUUCU
3240	ACUGAACU	U	UUGAGAUA	UAUCUCAA CUGAUGA X GAA AGUUCAGU

nt. Position	Target Sequence	Ribozyme Sequence
3241	CUGAACUU U UGAGAUAU	AUAUCUCA CUGAUGA X GAA AAGUUCAG
3242	UGAACUUU U GAGAUAUG	CAUAUCUC CUGAUGA X GAA AAAGUUCA
3248	UUUGAGAU A UGACGGUG	CACCGUCA CUGAUGA X GAA AUCUCAAA
3258	GACGGUGU A CUUACUGC	GCAGUAAG CUGAUGA X GAA ACACCGUC
3261	GGUGUACU U ACUGCCUU	AAGGCAGU CUGAUGA X GAA AGUACACC
3262	GUGUACUU A CUGCCUUG	CAAGGCAG CUGAUGA X GAA AAGUACAC
3269	UACUGCCU U GUAGCAAA	UUUGCUAC CUGAUGA X GAA AGGCAGUA
3272	UGCCUUGU A GCAAAAUA	UAUUUUGC CUGAUGA X GAA ACAAGGCA
3280	AGCAAAAU A AAGAUGUG	CACAUCUU CUGAUGA X GAA AUUUUGCU
3293	UGUGCCCU U AUUUUACC	GGUAAAAU CUGAUGA X GAA AGGGCACA
3294	GUGCCCUU A UUUUACCU	AGGUAAAA CUGAUGA X GAA AAGGGCAC

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20 3252). The length of stem II may be ≥ 2 base-pairs.

Table XV: Mouse c-myb Hammerhead Ribozyme and Target Sequence

nt. Position	Target Sequence	Ribozyme Sequence
10	CCGGGGCUC UUGGCGGA	UCCGCCAA CUGAUGA X GAA AGCCCCGG
12	GGGGCUCUU GGCGGAGC	GCUCCGCC CUGAUGA X GAA AGAGCCCC
33	GCCCGCCUC GCCAUGGC	GCCAUGGC CUGAUGA X GAA AGGCGGGC
63	CACAGCAUC UACAGUAG	CUACUGUA CUGAUGA X GAA AUGCUGUG
65	CAGCAUCUA CAGUAGCG	CGCUACUG CUGAUGA X GAA AGAUGCUG
70	UCUACAGUA GCGAUGAA	UUCAUCGC CUGAUGA X GAA ACUGUAGA
93	GAAGACAUU GAGAUGUG	CACAUCUC CUGAUGA X GAA AUGUCUUC
113	CCAUGACUA CGAUGGGC	GCCCAUCG CUGAUGA X GAA AGUCAUGG
134	GCCCAAAUC UGGAAAGC	GCUUUCCA CUGAUGA X GAA AUUUGGGC
145	GAAAGCGUC ACUUGGGG	CCCCAAGU CUGAUGA X GAA ACGCUUUC
149	GCGUCACUU GGGGAAAA	UUUUCCCC CUGAUGA X GAA AGUGACGC
160	GGAAAACUA GGUGGACA	UGUCCACC CUGAUGA X GAA AGUUUUCC
231	UGGAAAGUC AUUGCCAA	UUGGCAAU CUGAUGA X GAA ACUUUCCA
234	AAAGUCAUU GCCAAUUA	UAAUUGGC CUGAUGA X GAA AUGACUUU
241	UUGCCAAUU AUCUGCCC	GGGCAGAU CUGAUGA X GAA AUUGGCAA

nt. Position	Target Sequence	Ribozyme Sequence
242	UGCCAAUUA UCUGCCCA	UGGGCAGA CUGAUGA X GAA AAUUGGCA
244	CCAAUUAUC UGCCCAAC	GUUGGGCA CUGAUGA X GAA AUAAUUGG
264	ACAGAUGUA CAGUGCCA	UGGCACUG CUGAUGA X GAA ACAUCUGU
306	CCUGAACUC AUCAAAGG	CCUUUGAU CUGAUGA X GAA AGUUCAGG
309	GAACUCAUC AAAGGUCC	GGACCUUU CUGAUGA X GAA AUGAGUUC
316	UCAAAGGUC CCUGGACC	GGUCCAGG CUGAUGA X GAA ACCUUUGA
337	AAGAAGAUC AGAGAGUC	GACUCUCU CUGAUGA X GAA AUCUUCUU
345	CAGAGAGUC AUAAAGCU	AGCUUUAU CUGAUGA X GAA ACUCUCUG
348	AGAGUCAUA AAGCUUGU	ACAAGCUU CUGAUGA X GAA AUGACUCU
354	AUAAAGCUU GUCCAGAA	UUCUGGAC CUGAUGA X GAA AGCUUUAU
357	AAGCUUGUC CAGAAAUA	UAUUUCUG CUGAUGA X GAA ACAAGCUU
365	CCAGAAAUA UGGUCCGA	UCGGACCA CUGAUGA X GAA AUUUCUGG
370	AAUAUGGUC CGAAGCGU	ACGCUUCG CUGAUGA X GAA ACCAUAUU
379	CGAAGCGUU GGUCUGUU	AACAGACC CUGAUGA X GAA ACGCUUCG
383	GCGUUGGUC UGUUAUUG	CAAUAACA CUGAUGA X GAA ACCAACGC
387	UGGUCUGUU AUUGCCAA	UUGGCAAU CUGAUGA X GAA ACAGACCA
388	GGUCUGUUA UUGCCAAG	CUUGGCAA CUGAUGA X GAA AACAGACC
390	UCUGUUAUU GCCAAGCA	UGCUUGGC CUGAUGA X GAA AUAACAGA
401	CAAGCACUU AAAAGGGA	UCCCUUUU CUGAUGA X GAA AGUGCUUG
402	AAGCACUUA AAAGGGAG	CUCCCUUU CUGAUGA X GAA AAGUGCUU
414	GGGAGAAUU GGAAAGCA	UGCUUUCC CUGAUGA X GAA AUUCUCCC
427	AGCAGUGUC GGGAGAGG	CCUCUCCC CUGAUGA X GAA ACACUGCU
448	ACAACCAUU UGAAUCCA	UGGAUUCA CUGAUGA X GAA AUGGUUGU
449	CAACCAUUU GAAUCCAG	CUGGAUUC CUGAUGA X GAA AAUGGUUG
454	AUUUGAAUC CAGAAGUU	AACUUCUG CUGAUGA X GAA AUUCAAAU
462	CCAGAAGUU AAGAAAAC	GUUUUCUU CUGAUGA X GAA ACUUCUGG
463	CAGAAGUUA AGAAAACC	GGUUUUCU CUGAUGA X GAA AACUUCUG
473	GAAAACCUC CUGGACAG	CUGUCCAG CUGAUGA X GAA AGGUUUUC
498	GACAGAAUC AUUUACCA	UGGUAAAU CUGAUGA X GAA AUUCUGUC
501	AGAAUCAUU UACCAGGC	GCCUGGUA CUGAUGA X GAA AUGAUUCU
502	GAAUCAUUU ACCAGGCA	UGCCUGGU CUGAUGA X GAA AAUGAUUC
503	AAUCAUUUA CCAGGCAC	GUGCCUGG CUGAUGA X GAA AAAUGAUU
520	ACAAGCGUC UGGGGAAC	GUUCCCCA CUGAUGA X GAA ACGCUUGU
543	GCAGAGAUC GCAAAGCU	AGCUUUGC CUGAUGA X GAA AUCUCUGC
571	GGACUGAUA AUGCUAUC	GAUAGCAU CUGAUGA X GAA AUCAGUCC
577	AUAAUGCUA UCAAGAAC	GUUCUUGA CUGAUGA X GAA AGCAUUAU

nt. Position	Target Sequence	Ribozyme Sequence
579	AAUGCUAUC AAGAACCA	UGGUUCUU CUGAUGA X GAA AUAGCAUU
595	ACUGGAAUU CCACCAUG	CAUGGUGG CUGAUGA X GAA AUUCCAGU
596	CUGGAAUUC CACCAUGC	GCAUGGUG CUGAUGA X GAA AAUUCCAG
607	CCAUGCGUC GCAAGGUG	CACCUUGC CUGAUGA X GAA ACGCAUGG
629	GGAAGGCUA CCUGCAGA	UCUGCAGG CUGAUGA X GAA AGCCUUCC
643	AGAAGCCUU CCAAAGCC	GGCUUUGG CUGAUGA X GAA AGGCUUCU
644	GAAGCCUUC CAAAGCCA	UGGCUUUG CUGAUGA X GAA AAGGCUUC
677	CACGAGCUU CCAGAAGA	UCUUCUGG CUGAUGA X GAA AGCUCGUG
678	ACGAGCUUC CAGAAGAA	UUCUUCUG CUGAUGA X GAA AAGCUCGU
691	AGAACAAUC AUUUGAUG	CAUCAAAU CUGAUGA X GAA AUUGUUCU
694	ACAAUCAUU UGAUGGGG	CCCCAUCA CUGAUGA X GAA AUGAUUGU
695	CAAUCAUUU GAUGGGGU	ACCCCAUC CUGAUGA X GAA AAUGAUUG
704	GAUGGGGUU UGGGCAUG	CAUGCCCA CUGAUGA X GAA ACCCCAUC
705	AUGGGGUUU GGGCAUGC	GCAUGCCC CUGAUGA X GAA AACCCCAU
716	GCAUGCCUC ACCUCCAU	AUGGAGGU CUGAUGA X GAA AGGCAUGC
721	CCUCACCUC CAUCUCAG	CUGAGAUG CUGAUGA X GAA AGGUGAGG
725	ACCUCCAUC UCAGCUCU	AGAGCUGA CUGAUGA X GAA AUGGAGGU
727	CUCCAUCUC AGCUCUCU	AGAGAGCU CUGAUGA X GAA AGAUGGAG
732	UCUCAGCUC UCUCCAAG	CUUGGAGA CUGAUGA X GAA AGCUGAGA
734	UCAGCUCUC UCCAAGUG	CACUUGGA CUGAUGA X GAA AGAGCUGA
736	AGCUCUCUC CAAGUGGC	GCCACUUG CUGAUGA X GAA AGAGAGCU
749	UGGCCAGUC CUCCGUCA	UGACGGAG CUGAUGA X GAA ACUGGCCA
752	CCAGUCCUC CGUCAACA	UGUUGACG CUGAUGA X GAA AGGACUGG
756	UCCUCCGUC AACAGCGA	UCGCUGUU CUGAUGA X GAA ACGGAGGA
767	CAGCGAAUA UCCCUAUU	AAUAGGGA CUGAUGA X GAA AUUCGCUG
769	GCGAAUAUC CCUAUUAC	GUAAUAGG CUGAUGA X GAA AUAUUCGC
773	AUAUCCCUA UUACCACA	UGUGGUAA CUGAUGA X GAA AGGGAUAU
775	AUCCCUAUU ACCACAUC	GAUGUGGU CUGAUGA X GAA AUAGGGAU
776	UCCCUAUUA CCACAUCG	CGAUGUGG CUGAUGA X GAA AAUAGGGA
783	UACCACAUC GCCGAAGC	GCUUCGGC CUGAUGA X GAA AUGUGGUA
801	CAAAACAUC UCCAGUCA	UGACUGGA CUGAUGA X GAA AUGUUUUG
803	AAACAUCUC CAGUCACG	CGUGACUG CUGAUGA X GAA AGAUGUUU
808	UCUCCAGUC ACGUUCCC	GGGAACGU CUGAUGA X GAA ACUGGAGA
813	AGUCACGUU CCCUAUCC	GGAUAGGG CUGAUGA X GAA ACGUGACU
814	GUCACGUUC CCUAUCCU	AGGAUAGG CUGAUGA X GAA AACGUGAC
818	CGUUCCCUA UCCUGUCG	CGACAGGA CUGAUGA X GAA AGGGAACG

nt. Position	Target Sequence	Ribozyme Sequence
820	UUCCCUAUC CUGUCGCA	UGCGACAG CUGAUGA X GAA AUAGGGAA
825	UAUCCUGUC GCAUUGCA	UGCAAUGC CUGAUGA X GAA ACAGGAUA
830	UGUCGCAUU GCAUGUUA	UAACAUGC CUGAUGA X GAA AUGCGACA
837	UUGCAUGUU AAUAUAGU	ACUAUAUU CUGAUGA X GAA ACAUGCAA
838	UGCAUGUUA AUAUAGUC	GACUAUAU CUGAUGA X GAA AACAUGCA
841	AUGUUAAUA UAGUCAAC	GUUGACUA CUGAUGA X GAA AUUAACAU
843	GUUAAUAUA GUCAACGU	ACGUUGAC CUGAUGA X GAA AUAUUAAC
846	AAUAUAGUC AACGUCCC	GGGACGUU CUGAUGA X GAA ACUAUAUU
852	GUCAACGUC CCUCAGCC	GGCUGAGG CUGAUGA X GAA ACGUUGAC
856	ACGUCCCUC AGCCGGCU	AGCCGGCU CUGAUGA X GAA AGGGACGU
876	GCAGCCAUC CAGAGACA	UGUCUCUG CUGAUGA X GAA AUGGCUGC
887	GAGACACUA UAACGACG	CGUCGUUA CUGAUGA X GAA AGUGUCUC
889	GACACUAUA ACGACGAA	UUCGUCGU CUGAUGA X GAA AUAGUGUC
921	AAGCGAAUA AAGGAGCU	AGCUCCUU CUGAUGA X GAA AUUCGCUU
935	GCUGGAGUU GCUCCUGA	UCAGGAGC CUGAUGA X GAA ACUCCAGC
939	GAGUUGCUC CUGAUGUC	GACAUCAG CUGAUGA X GAA AGCAACUC
947	CCUGAUGUC AACAGAGA	UCUCUGUU CUGAUGA X GAA ACAUCAGG
980	GCAGGCAUU ACCAACAC	GUGUUGGU CUGAUGA X GAA AUGCCUGC
981	CAGGCAUUA CCAACACA	UGUGUUGG CUGAUGA X GAA AAUGCCUG
1000	ACCACACUU GCAGCUAC	GUAGCUGC CUGAUGA X GAA AGUGUGGU
1007	UUGCAGCUA CCCCGGGU	ACCCGGGG CUGAUGA X GAA AGCUGCAA
1028	CAGCACCUC CAUUGUGG	CCACAAUG CUGAUGA X GAA AGGUGCUG
1032	ACCUCCAUU GUGGACCA	UGGUCCAC CUGAUGA X GAA AUGGAGGU
1051	CCAGACCUC AUGGGGAU	AUCCCCAU CUGAUGA X GAA AGGUCUGG
1060	AUGGGGAUA GUGCACCU	AGGUGCAC CUGAUGA X GAA AUCCCCAU
1071	GCACCUGUU UCCUGUUU	AAACAGGA CUGAUGA X GAA ACAGGUGC
1072	CACCUGUUU CCUGUUUG	CAAACAGG CUGAUGA X GAA AACAGGUG
1073	ACCUGUUUC CUGUUUGG	CCAAACAG CUGAUGA X GAA AAACAGGU
1078	UUUCCUGUU UGGGAGAA	UUCUCCCA CUGAUGA X GAA ACAGGAAA
1079	UUCCUGUUU GGGAGAAC	GUUCUCCC CUGAUGA X GAA AACAGGAA
1103	CACCCCAUC UCUGCCUG	CAGGCAGA CUGAUGA X GAA AUGGGGUG
1105	CCCCAUCUC UGCCUGCA	UGCAGGCA CUGAUGA X GAA AGAUGGGG
1117	CUGCAGAUC CCGGCUCC	GGAGCCGG CUGAUGA X GAA AUCUGCAG
1124	UCCCGGCUC CCUACCUG	CAGGUAGG CUGAUGA X GAA AGCCGGGA
1128	GGCUCCCUA CCUGAAGA	UCUUCAGG CUGAUGA X GAA AGGGAGCC
1145	AAGUGCCUC ACCAGCAA	UUGCUGGU CUGAUGA X GAA AGGCACUU

nt. Position	Target Sequence	Ribozyme Sequence
1164	UGCAUGAUC GUCCACCA	UGGUGGAC CUGAUGA X GAA AUCAUGCA
1167	AUGAUCGUC CACCAGGG	CCCUGGUG CUGAUGA X GAA ACGAUCAU
1182	GGCACCAUU CUGGACAA	UUGUCCAG CUGAUGA X GAA AUGGUGCC
1183	GCACCAUUC UGGACAAU	AUUGUCCA CUGAUGA X GAA AAUGGUGC
1194	GACAAUGUU AAGAACCU	AGGUUCUU CUGAUGA X GAA ACAUUGUC
1195	ACAAUGUUA AGAACCUC	GAGGUUCU CUGAUGA X GAA AACAUUGU
1203	AAGAACCUC UUAGAAUU	AAUUCUAA CUGAUGA X GAA AGGUUCUU
1205	GAACCUCUU AGAAUUUG	CAAAUUCU CUGAUGA X GAA AGAGGUUC
1206	AACCUCUUA GAAUUUGC	GCAAAUUC CUGAUGA X GAA AAGAGGUU
1211	CUUAGAAUU UGCAGAAA	UUUCUGCA CUGAUGA X GAA AUUCUAAG
1212	UUAGAAUUU GCAGAAAC	GUUUCUGC CUGAUGA X GAA AAUUCUAA
1224	GAAACACUC CAGUUUAU	AUAAACUG CUGAUGA X GAA AGUGUUUC
1229	ACUCCAGUU UAUAGAUU	AAUCUAUA CUGAUGA X GAA ACUGGAGU
1230	CUCCAGUUU AUAGAUUC	GAAUCUAU CUGAUGA X GAA AACUGGAG
1231	UCCAGUUUA UAGAUUCU	AGAAUCUA CUGAUGA X GAA AAACUGGA
1233	CAGUUUAUA GAUUCUUU	AAAGAAUC CUGAUGA X GAA AUAAACUG
1237	UUAUAGAUU CUUUCUUG	CAAGAAAG CUGAUGA X GAA AUCUAUAA
1238	UAUAGAUUC UUUCUUGA	UCAAGAAA CUGAUGA X GAA AAUCUAUA
1240	UAGAUUCUU UCUUGAAC	GUUCAAGA CUGAUGA X GAA AGAAUCUA
1241	AGAUUCUUU CUUGAACA	UGUUCAAG CUGAUGA X GAA AAGAAUCU
1242	GAUUCUUUC UUGAACAC	GUGUUCAA CUGAUGA X GAA AAAGAAUC
1244	UUCUUUCUU GAACACUU	AAGUGUUC CUGAUGA X GAA AGAAAGAA
1252	UGAACACUU CCAGCAAC	GUUGCUGG CUGAUGA X GAA AGUGUUCA
1253	GAACACUUC CAGCAACC	GGUUGCUG CUGAUGA X GAA AAGUGUUC
1271	UGAAAACUC GGGCUUAG	CUAAGCCC CUGAUGA X GAA AGUUUUCA
1277	CUCGGGCUU AGAUGCAC	GUGCAUCU CUGAUGA X GAA AGCCCGAG
1278	UCGGGCUUA GAUGCACC	GGUGCAUC CUGAUGA X GAA AAGCCCGA
1288	AUGCACCUA CCUUACCC	GGGUAAGG CUGAUGA X GAA AGGUGCAU
1292	ACCUACCUU ACCCUCCA	UGGAGGGU CUGAUGA X GAA AGGUAGGU
1293	CCUACCUUA CCCUCCAC	GUGGAGGG CUGAUGA X GAA AAGGUAGG
1298	CUUACCCUC CACUCCUC	GAGGAGUG CUGAUGA X GAA AGGGUAAG
1303	CCUCCACUC CUCUCAUU	AAUGAGAG CUGAUGA X GAA AGUGGAGG
1306	CCACUCCUC UCAUUGGU	ACCAAUGA CUGAUGA X GAA AGGAGUGG
1308	ACUCCUCUC AUUGGUCA	UGACCAAU CUGAUGA X GAA AGAGGAGU
1311	CCUCUCAUU GGUCACAA	UUGUGACC CUGAUGA X GAA AUGAGAGG
1315	UCAUUGGUC ACAAACUG	CAGUUUGU CUGAUGA X GAA ACCAAUGA

nt. Position	Target Sequence	Ribozyme Sequence
1333	CACCAUGUC GAGACCAG	CUGGUCUC CUGAUGA X GAA ACAUGGUG
1366	AGGAAAAUU CCAUCUUU	AAAGAUGG CUGAUGA X GAA AUUUUCCU
1367	GGAAAAUUC CAUCUUUA	UAAAGAUG CUGAUGA X GAA AAUUUUCC
1371	AAUUCCAUC UUUAGAAC	GUUCUAAA CUGAUGA X GAA AUGGAAUU
1373	UUCCAUCUU UAGAACUC	GAGUUCUA CUGAUGA X GAA AGAUGGAA
1374	UCCAUCUUU AGAACUCC	GGAGUUCU CUGAUGA X GAA AAGAUGGA
1375	CCAUCUUUA GAACUCCA	UGGAGUUC CUGAUGA X GAA AAAGAUGG
1381	UUAGAACUC CAGCUAUC	GAUAGCUG CUGAUGA X GAA AGUUCUAA
1387	CUCCAGCUA UCAAAAGG	CCUUUUGA CUGAUGA X GAA AGCUGGAG
1389	CCAGCUAUC AAAAGGUC	GACCUUUU CUGAUGA X GAA AUAGCUGG
1397	CAAAAGGUC AAUCCUCG	CGAGGAUU CUGAUGA X GAA ACCUUUUG
1401	AGGUCAAUC CUCGAAAG	CUUUCGAG CUGAUGA X GAA AUUGACCU
1404	UCAAUCCUC GAAAGCUC	GAGCUUUC CUGAUGA X GAA AGGAUUGA
1412	CGAAAGCUC UCCUCGAA	UUCGAGGA CUGAUGA X GAA AGCUUUCG
1414	AAAGCUCUC CUCGAACU	AGUUCGAG CUGAUGA X GAA AGAGCUUU
1417	GCUCUCCUC GAACUCCC	GGGAGUUC CUGAUGA X GAA AGGAGAGC
1423	CUCGAACUC CCACACCA	UGGUGUGG CUGAUGA X GAA AGUUCGAG
1433	CACACCAUU CAAACAUG	CAUGUUUG CUGAUGA X GAA AUGGUGUG
1434	ACACCAUUC AAACAUGC	GCAUGUUU CUGAUGA X GAA AAUGGUGU
1446	CAUGCCCUU GCAGCUCA	UGAGCUGC CUGAUGA X GAA AGGGCAUG
1453	UUGCAGCUC AAGAAAUU	AAUUUCUU CUGAUGA X GAA AGCUGCAA
1461	CAAGAAAUU AAAUACGG	CCGUAUUU CUGAUGA X GAA AUUUCUUG
1462	AAGAAAUUA AAUACGGU	ACCGUAUU CUGAUGA X GAA AAUUUCUU
1466	AAUUAAAUA CGGUCCCC	GGGGACCG CUGAUGA X GAA AUUUAAUU
1471	AAUACGGUC CCCUGAAG	CUUCAGGG CUGAUGA X GAA ACCGUAUU
1485	AAGAUGCUA CCUCAGAC	GUCUGAGG CUGAUGA X GAA AGCAUCUU
1489	UGCUACCUC AGACCCCC	GGGGGUCU CUGAUGA X GAA AGGUAGCA
1499	GACCCCCUC CCAUGCAG	CUGCAUGG CUGAUGA X GAA AGGGGGUC
1518	GAGGACCUA CAAGAUGU	ACAUCUUG CUGAUGA X GAA AGGUCCUC
1530	GAUGUGAUU AAGCGGGA	UCCCGCUU CUGAUGA X GAA AUCACAUC
1531	AUGUGAUUA AGCGGGAA	UUCCCGCU CUGAUGA X GAA AAUCACAU
1541	GCGGGAAUC GGAUGAAU	AUUCAUCC CUGAUGA X GAA AUUCCCGC
1550	GGAUGAAUC UGGAAUUG	CAAUUCCA CUGAUGA X GAA AUUCAUCC
1557	UCUGGAAUU GUUGCUGA	UCAGCAAC CUGAUGA X GAA AUUCCAGA
1560	GGAAUUGUU GCUGAGUU	AACUCAGC CUGAUGA X GAA ACAAUUCC
1568	UGCUGAGUU UCAAGAGA	UCUCUUGA CUGAUGA X GAA ACUCAGCA

nt. Position	Target Sequence	Ribozyme Sequence
1569	GCUGAGUUU CAAGAGAG	CUCUCUUG CUGAUGA X GAA AACUCAGC
1570	CUGAGUUUC AAGAGAGU	ACUCUCUU CUGAUGA X GAA AAACUCAG
1589	ACCACCGUU ACUGAAAA	UUUUCAGU CUGAUGA X GAA ACGGUGGU
1590	CCACCGUUA CUGAAAAA	UUUUUCAG CUGAUGA X GAA AACGGUGG
1602	AAAAAAAUC AAGCAGGC	GCCUGCUU CUGAUGA X GAA AUUUUUUU
1619	GGUGGAGUC GCCAACUG	CAGUUGGC CUGAUGA X GAA ACUCCACC
1634	UGAGAAAUC GGGAAACU	AGUUUCCC CUGAUGA X GAA AUUUCUCA
1643	GGGAAACUU CUUCUGCU	AGCAGAAG CUGAUGA X GAA AGUUUCCC
1644	GGAAACUUC UUCUGCUC	GAGCAGAA CUGAUGA X GAA AAGUUUCC
1646	AAACUUCUU CUGCUCAA	UUGAGCAG CUGAUGA X GAA AGAAGUUU
1647	AACUUCUUC UGCUCAAA	UUUGAGCA CUGAUGA X GAA AAGAAGUU
1652	CUUCUGCUC AAACCACU	AGUGGUUU CUGAUGA X GAA AGCAGAAG
1691	CCAACUGUU CUCGCAGG	CCUGCGAG CUGAUGA X GAA ACAGUUGG
1692	CAACUGUUC UCGCAGGC	GCCUGCGA CUGAUGA X GAA AACAGUUG
1694	ACUGUUCUC GCAGGCGU	ACGCCUGC CUGAUGA X GAA AGAACAGU
1703	GCAGGCGUC UCCUGUGG	CCACAGGA CUGAUGA X GAA ACGCCUGC
1705	AGGCGUCUC CUGUGGCA	UGCCACAG CUGAUGA X GAA AGACGCCU
1726	CCCCAAAUA UUCUUACA	UGUAAGAA CUGAUGA X GAA AUUUGGGG
1728	CCAAAUAUU CUUACAAG	CUUGUAAG CUGAUGA X GAA AUAUUUGG
1729	CAAAUAUUC UUACAAGC	GCUUGUAA CUGAUGA X GAA AAUAUUUG
1731	AAUAUUCUU ACAAGCUC	GAGCUUGU CUGAUGA X GAA AGAAUAUU
1732	AUAUUCUUA CAAGCUCU	AGAGCUUG CUGAUGA X GAA AAGAAUAU
1739	UACAAGCUC UGUUUUAA	UUAAAACA CUGAUGA X GAA AGCUUGUA
1743	AGCUCUGUU UUAAUGAC	GUCAUUAA CUGAUGA X GAA ACAGAGCU
1744	GCUCUGUUU UAAUGACA	UGUCAUUA CUGAUGA X GAA AACAGAGC
1745	CUCUGUUUU AAUGACAC	GUGUCAUU CUGAUGA X GAA AAACAGAG
1746	UCUGUUUUA AUGACACC	GGUGUCAU CUGAUGA X GAA AAAACAGA
1758	ACACCUGUA UCAGAAGA	UCUUCUGA CUGAUGA X GAA ACAGGUGU
1760	ACCUGUAUC AGAAGAUG	CAUCUUCU CUGAUGA X GAA AUACAGGU
1779	GACAAUGUC CUCAAAGC	GCUUUGAG CUGAUGA X GAA ACAUUGUC
1782	AAUGUCCUC AAAGCCUU	AAGGCUUU CUGAUGA X GAA AGGACAUU
1790	CAAAGCCUU UACCGUAC	GUACGGUA CUGAUGA X GAA AGGCUUUG
1791	AAAGCCUUU ACCGUACC	GGUACGGU CUGAUGA X GAA AAGGCUUU
1792	AAGCCUUUA CCGUACCU	AGGUACGG CUGAUGA X GAA AAAGGCUU
1797	UUUACCGUA CCUAAGAA	UUCUUAGG CUGAUGA X GAA ACGGUAAA
1801	CCGUACCUA AGAACAGG	CCUGUUCU CUGAUGA X GAA AGGUACGG

nt. Position	Target Sequence	Ribozyme Sequence
1822	UGGUGGGUC CCUUGCAG	CUGCAAGG CUGAUGA X GAA ACCCACCA
1826	GGGUCCCUU GCAGCCAU	AUGGCUGC CUGAUGA X GAA AGGGACCC
1859	GCCAGCAUC CUGUGGGA	UCCCACAG CUGAUGA X GAA AUGCUGGC
1892	GACGGCCUC CGGUCCGG	CCGGACCG CUGAUGA X GAA AGGCCGUC
1897	CCUCCGGUC CGGCUCGG	CCGAGCCG CUGAUGA X GAA ACCGGAGG
1903	GUCCGGCUC GGAAAUAC	GUAUUUCC CUGAUGA X GAA AGCCGGAC
1910	UCGGAAAUA CGUGAACG	CGUUCACG CUGAUGA X GAA AUUUCCGA
1922	GAACGCGUU CUCAGCUC	GAGCUGAG CUGAUGA X GAA ACGCGUUC
1923	AACGCGUUC UCAGCUCG	CGAGCUGA CUGAUGA X GAA AACGCGUU
1925	CGCGUUCUC AGCUCGAA	UUCGAGCU CUGAUGA X GAA AGAACGCG
1930	UCUCAGCUC GAACUCUG	CAGAGUUC CUGAUGA X GAA AGCUGAGA
1936	CUCGAACUC UGGUCAUG	CAUGACCA CUGAUGA X GAA AGUUCGAG
1941	ACUCUGGUC AUGUGAGA	UCUCACAU CUGAUGA X GAA ACCAGAGU
1953	UGAGACAUU UCCAGAAA	UUUCUGGA CUGAUGA X GAA AUGUCUCA
1954	GAGACAUUU CCAGAAAA	UUUUCUGG CUGAUGA X GAA AAUGUCUC
1955	AGACAUUUC CAGAAAAG	CUUUUCUG CUGAUGA X GAA AAAUGUCU
1967	AAAAGCAUU AUGGUUUU	AAAACCAU CUGAUGA X GAA AUGCUUUU
1968	AAAGCAUUA UGGUUUUC	GAAAACCA CUGAUGA X GAA AAUGCUUU
1973	AUUAUGGUU UUCAGAAC	GUUCUGAA CUGAUGA X GAA ACCAUAAU
1974	UUAUGGUUU UCAGAACA	UGUUCUGA CUGAUGA X GAA AACCAUAA
1975	UAUGGUUUU CAGAACAC	GUGUUCUG CUGAUGA X GAA AAACCAUA
1976	AUGGUUUUC AGAACACU	AGUGUUCU CUGAUGA X GAA AAAACCAU
1985	AGAACACUU AAAAGUUG	CAACUUUU CUGAUGA X GAA AGUGUUCU
1986	GAACACUUA AAAGUUGA	UCAACUUU CUGAUGA X GAA AAGUGUUC
1992	UUAAAAGUU GACUUUCG	CGAAAGUC CUGAUGA X GAA ACUUUUAA
1997	AGUUGACUU UCGACACA	UGUGUCGA CUGAUGA X GAA AGUCAACU
1998	GUUGACUUU CGACACAU	AUGUGUCG CUGAUGA X GAA AAGUCAAC
1999	UUGACUUUC GACACAUG	CAUGUGUC CUGAUGA X GAA AAAGUCAA
2011	ACAUGGCUC CUCAGCGU	ACGCUGAG CUGAUGA X GAA AGCCAUGU
2014	UGGCUCCUC AGCGUGGA	UCCACGCU CUGAUGA X GAA AGGAGCCA
2028	GGAGCGCUC CAUGGCUG	CAGCCAUG CUGAUGA X GAA AGCGCUCC
2052	AGCCUGAUU UUGUUGUG	CACAACAA CUGAUGA X GAA AUCAGGCU
2053	GCCUGAUUU UGUUGUGG	CCACAACA CUGAUGA X GAA AAUCAGGC
2054	CCUGAUUUU GUUGUGGU	ACCACAAC CUGAUGA X GAA AAAUCAGG
2057	GAUUUUGUU GUGGUACA	UGUACCAC CUGAUGA X GAA ACAAAAUC
2063	GUUGUGGUA CAACAGUU	AACUGUUG CUGAUGA X GAA ACCACAAC

nt. Position	Target Sequence	Ribozyme Sequence
2071	ACAACAGUU GAGAGCAG	CUGCUCUC CUGAUGA X GAA ACUGUUGU
2092	AAGUGCAUU UUUAGUUG	CAACUAAA CUGAUGA X GAA AUGCACUU
2093	AGUGCAUUU UUAGUUGC	GCAACUAA CUGAUGA X GAA AAUGCACU
2094	GUGCAUUUU UAGUUGCU	AGCAACUA CUGAUGA X GAA AAAUGCAC
2095	UGCAUUUUU AGUUGCUU	AAGCAACU CUGAUGA X GAA AAAAUGCA
2096	GCAUUUUUA GUUGCUUG	CAAGCAAC CUGAUGA X GAA AAAAAUGC
2099	UUUUUAGUU GCUUGAGA	UCUCAAGC CUGAUGA X GAA ACUAAAAA
2103	UAGUUGCUU GAGAUCUC	GAGAUCUC CUGAUGA X GAA AGCAACUA
2109	CUUGAGAUC UCACUUGA	UCAAGUGA CUGAUGA X GAA AUCUCAAG
2111	UGAGAUCUC ACUUGAUU	AAUCAAGU CUGAUGA X GAA AGAUCUCA
2115	AUCUCACUU GAUUUCAC	GUGAAAUC CUGAUGA X GAA AGUGAGAU
2119	CACUUGAUU UCACACAA	UUGUGUGA CUGAUGA X GAA AUCAAGUG
2120	ACUUGAUUU CACACAAC	GUUGUGUG CUGAUGA X GAA AAUCAAGU
2121	CUUGAUUUC ACACAACU	AGUUGUGU CUGAUGA X GAA AAAUCAAG
2130	ACACAACUA AAAAGGAU	AUCCUUUU CUGAUGA X GAA AGUUGUGU
2139	AAAAGGAUU UUUUUUUU	AAAAAAAA CUGAUGA X GAA AUCCUUUU
2140	AAAGGAUUU UUUUUUUA	UAAAAAAA CUGAUGA X GAA AAUCCUUU
2141	AAGGAUUUU UUUUUUAA	UUAAAAAA CUGAUGA X GAA AAAUCCUU
2142	AGGAUUUUU UUUUUAAA	UUUAAAAA CUGAUGA X GAA AAAAUCCU
2143	GGAUUUUUU UUUUAAAA	UUUUAAAA CUGAUGA X GAA AAAAAUCC
2144	GAUUUUUUU UUUAAAAA	UUUUUAAA CUGAUGA X GAA AAAAAAUC
2145	AUUUUUUUU UUAAAAAU	AUUUUUAA CUGAUGA X GAA AAAAAAAU
2146	UUUUUUUUU UAAAAAUA	UAUUUUUA CUGAUGA X GAA AAAAAAAA
2147	UUUUUUUUU AAAAAUAA	UUAUUUUU CUGAUGA X GAA AAAAAAAA
2148	UUUUUUUUA AAAAUAAU	AUUAUUUU CUGAUGA X GAA AAAAAAAA
2154	UUAAAAAUA AUAAUAAU	AUUAUUAU CUGAUGA X GAA AUUUUUAA
2157	AAAAUAAUA AUAAUGAA	UUCAUUAU CUGAUGA X GAA AUUAUUUU
2160	AUAAUAAUA AUGAAUAA	UUAUUCAU CUGAUGA X GAA AUUAUUAU
2167	UAAUGAAUA ACAGUCUU	AAGACUGU CUGAUGA X GAA AUUCAUUA
2173	AUAACAGUC UUACCUAA	UUAGGUAA CUGAUGA X GAA ACUGUUAU
2175	AACAGUCUU ACCUAAAU	AUUUAGGU CUGAUGA X GAA AGACUGUU
2176	ACAGUCUUA CCUAAAUU	AAUUUAGG CUGAUGA X GAA AAGACUGU
2180	UCUUACCUA AAUUAUUA	UAAUAAUU CUGAUGA X GAA AGGUAAGA
2184	ACCUAAAUU AUUAGGUA	UACCUAAU CUGAUGA X GAA AUUUAGGU
2185	CCUAAAUUA UUAGGUAA	UUACCUAA CUGAUGA X GAA AAUUUAGG
2187	UAAAUUAUU AGGUAAUG	CAUUACCU CUGAUGA X GAA AUAAUUUA

nt. Position	Target Sequence	Ribozyme Sequence
2188	AAAUUAUUA GGUAAUGA	UCAUUACC CUGAUGA X GAA AAUAAUUU
2192	UAUUAGGUA AUGAAUUG	CAAUUCAU CUGAUGA X GAA ACCUAAUA
2199	UAAUGAAUU GUGACCAU	AUGGUCAC CUGAUGA X GAA AUUCAUUA
2208	GUGACCAUU UGUUAAUA	UAUUAACA CUGAUGA X GAA AUGGUCAC
2209	UGACCAUUU GUUAAUAU	AUAUUAAC CUGAUGA X GAA AAUGGUCA
2212	CCAUUUGUU AAUAUCAU	AUGAUAUU CUGAUGA X GAA ACAAAUGG
2213	CAUUUGUUA AUAUCAUA	UAUGAUAU CUGAUGA X GAA AACAAAUG
2216	UUGUUAAUA UCAUAAUC	GAUUAUGA CUGAUGA X GAA AUUAACAA
2218	GUUAAUAUC AUAAUCAG	CUGAUUAU CUGAUGA X GAA AUAUUAAC
2221	AAUAUCAUA AUCAGAUU	AAUCUGAU CUGAUGA X GAA AUGAUAUU
2224	AUCAUAAUC AGAUUUUU	AAAAAUCU CUGAUGA X GAA AUUAUGAU
2229	AAUCAGAUU UUUUAAAA	UUUUAAAA CUGAUGA X GAA AUCUGAUU
2230	AUCAGAUUU UUUAAAAA	UUUUUAAA CUGAUGA X GAA AAUCUGAU
2231	UCAGAUUUU UUAAAAAA	UUUUUUAA CUGAUGA X GAA AAAUCUGA
2232	CAGAUUUUU UAAAAAAA	UUUUUUUA CUGAUGA X GAA AAAAUCUG
2233	AGAUUUUUU AAAAAAAA	UUUUUUUU CUGAUGA X GAA AAAAAUCU
2234	GAUUUUUUA AAAAAAAU	AUUUUUUU CUGAUGA X GAA AAAAAAUC
2243	AAAAAAAUA AAAUGAUU	AAUCAUUU CUGAUGA X GAA AUUUUUUU
2251	AAAAUGAUU UAUUUGUA	UACAAAUA CUGAUGA X GAA AUCAUUUU
2252	AAAUGAUUU AUUUGUAU	AUACAAAU CUGAUGA X GAA AAUCAUUU
2253	AAUGAUUUA UUUGUAUU	AAUACAAA CUGAUGA X GAA AAAUCAUU
2255	UGAUUUAUU UGUAUUUU	AAAAUACA CUGAUGA X GAA AUAAAUCA
2256	GAUUUAUUU GUAUUUUA	UAAAAUAC CUGAUGA X GAA AAUAAAUC
2259	UUAUUUGUA UUUUAGAG	CUCUAAAA CUGAUGA X GAA ACAAAUAA
2261	AUUUGUAUU UUAGAGAA	UUCUCUAA CUGAUGA X GAA AUACAAAU
2262	UUUGUAUUU UAGAGAAU	AUUCUCUA CUGAUGA X GAA AAUACAAA
2263	UUGUAUUUU AGAGAAUA	UAUUCUCU CUGAUGA X GAA AAAUACAA
2264	UGUAUUUUA GAGAAUAC	GUAUUCUC CUGAUGA X GAA AAAAUACA
2271	UAGAGAAUA CAACAGAU	AUCUGUUG CUGAUGA X GAA AUUCUCUA
2280	CAACAGAUC AGUAUUUU	AAAAUACU CUGAUGA X GAA AUCUGUUG
2284	AGAUCAGUA UUUUUGAC	GUCAAAAA CUGAUGA X GAA ACUGAUCU
2286	AUCAGUAUU UUUGACUG	CAGUCAAA CUGAUGA X GAA AUACUGAU
2287	UCAGUAUUU UUGACUGU	ACAGUCAA CUGAUGA X GAA AAUACUGA
2288	CAGUAUUUU UGACUGUG	CACAGUCA CUGAUGA X GAA AAAUACUG
2289	AGUAUUUUU GACUGUGG	CCACAGUC CUGAUGA X GAA AAAAUACU
2303	UGGUGAAUU UAAAAAAA	UUUUUUUA CUGAUGA X GAA AUUCACCA

nt. Position	Target Sequence	Ribozyme Sequence
2304	GGUGAAUUU AAAAAAA	UUUUUUUU CUGAUGA X GAA AAUUCACC
2305	GUGAAUUUA AAAAAAA	UUUUUUUU CUGAUGA X GAA AAAUUCAC
2316	AAAAAAAUU UACACAAA	UUUGUGUA CUGAUGA X GAA AUUUUUUU
2317	AAAAAAUUU ACACAAAG	CUUUGUGU CUGAUGA X GAA AAUUUUUU
2318	AAAAAUUUA CACAAAGA	UCUUUGUG CUGAUGA X GAA AAAUUUUU
2330	AAAGAAAUA UCCCAGUA	UACUGGGA CUGAUGA X GAA AUUUCUUU
2332	AGAAAUAUC CCAGUAUU	AAUACUGG CUGAUGA X GAA AUAUUUCU
2338	AUCCCAGUA UUCCAUGU	ACAUGGAA CUGAUGA X GAA ACUGGGAU
2340	CCCAGUAUU CCAUGUAU	AUACAUGG CUGAUGA X GAA AUACUGGG
2341	CCAGUAUUC CAUGUAUC	GAUACAUG CUGAUGA X GAA AAUACUGG
2347	UUCCAUGUA UCUCAGUC	GACUGAGA CUGAUGA X GAA ACAUGGAA
2349	CCAUGUAUC UCAGUCAC	GUGACUGA CUGAUGA X GAA AUACAUGG
2351	AUGUAUCUC AGUCACUA	UAGUGACU CUGAUGA X GAA AGAUACAU
2355	AUCUCAGUC ACUAAACA	UGUUUAGU CUGAUGA X GAA ACUGAGAU
2359	CAGUCACUA AACAUACA	UGUAUGUU CUGAUGA X GAA AGUGACUG
2365	CUAAACAUA CACAGAGA	UCUCUGUG CUGAUGA X GAA AUGUUUAG
2377	AGAGAGAUU UUUAAAAA	UUUUUAAA CUGAUGA X GAA AUCUCUCU
2378	GAGAGAUUU UUAAAAAC	GUUUUUAA CUGAUGA X GAA AAUCUCUC
2379	AGAGAUUUU UAAAAACC	GGUUUUUA CUGAUGA X GAA AAAUCUCU
2380	GAGAUUUUU AAAAACCA	UGGUUUUU CUGAUGA X GAA AAAAUCUC
2381	AGAUUUUUA AAAACCAG	CUGGUUUU CUGAUGA X GAA AAAAAUCU
2399	AGAAGCAUU AUUUUGAA	UUCAAAAU CUGAUGA X GAA AUGCUUCU
2400	GAAGCAUUA UUUUGAAU	AUUCAAAA CUGAUGA X GAA AAUGCUUC
2402	AGCAUUAUU UUGAAUGU	ACAUUCAA CUGAUGA X GAA AUAAUGCU
2403	GCAUUAUUU UGAAUGUU	AACAUUCA CUGAUGA X GAA AAUAAUGC
2404	CAUUAUUUU GAAUGUUA	UAACAUUC CUGAUGA X GAA AAAUAAUG
2411	UUGAAUGUU AGCUAAAU	AUUUAGCU CUGAUGA X GAA ACAUUCAA
2412	UGAAUGUUA GCUAAAUC	GAUUUAGC CUGAUGA X GAA AACAUUCA
2416	UGUUAGCUA AAUCCCAA	UUGGGAUU CUGAUGA X GAA AGCUAACA
2420	AGCUAAAUC CCAAGUAA	UUACUUGG CUGAUGA X GAA AUUUAGCU
2427	UCCCAAGUA AUACUUAA	UUAAGUAU CUGAUGA X GAA ACUUGGGA
2430	CAAGUAAUA CUUAAUGC	GCAUUAAG CUGAUGA X GAA AUUACUUG
2433	GUAAUACUU AAUGCAAC	GUUGCAUU CUGAUGA X GAA AGUAUUAC
2434	UAAUACUUA AUGCAACC	GGUUGCAU CUGAUGA X GAA AAGUAUUA
2445	GCAACCCUC UAGGAGCU	AGCUCCUA CUGAUGA X GAA AGGGUUGC
2447	AACCCUCUA GGAGCUCA	UGAGCUCC CUGAUGA X GAA AGAGGGUU

nt. Position	Target Sequence	Ribozyme Sequence
2454	UAGGAGCUC AUUUGUGG	CCACAAAU CUGAUGA X GAA AGCUCCUA
2457	GAGCUCAUU UGUGGCUA	UAGCCACA CUGAUGA X GAA AUGAGCUC
2458	AGCUCAUUU GUGGCUAA	UUAGCCAC CUGAUGA X GAA AAUGAGCU
2465	UUGUGGCUA AUAAUCUU	AAGAUUAU CUGAUGA X GAA AGCCACAA
2468	UGGCUAAUA AUCUUGGA	UCCAAGAU CUGAUGA X GAA AUUAGCCA
2471	CUAAUAAUC UUGGAAAU	AUUUCCAA CUGAUGA X GAA AUUAUUAG
2473	AAUAAUCUU GGAAAUAU	AUAUUUCC CUGAUGA X GAA AGAUUAUU
2480	UUGGAAAUA UCUUUAUU	AAUAAAGA CUGAUGA X GAA AUUUCCAA
2482	GGAAAUAUC UUUAUUAU	AUAAUAAA CUGAUGA X GAA AUAUUUCC
2484	AAAUAUCUU UAUUAUAU	AUAUAAUA CUGAUGA X GAA AGAUAUUU
2485	AAUAUCUUU AUUAUAUA	UAUAUAAU CUGAUGA X GAA AAGAUAUU
2486	AUAUCUUUA UUAUAUAG	CUAUAUAA CUGAUGA X GAA AAAGAUAU
2488	AUCUUUAUU AUAUAGCA	UGCUAUAU CUGAUGA X GAA AUAAAGAU
2489	UCUUUAUUA UAUAGCAU	AUGCUAUA CUGAUGA X GAA AAUAAAGA
2491	UUUAUUAUA UAGCAUUU	AAAUGCUA CUGAUGA X GAA AUAAUAAA
2493	UAUUAUAUA GCAUUUAU	AUAAAUGC CUGAUGA X GAA AUAUAAUA
2498	UAUAGCAUU UAUGAGGA	UCCUCAUA CUGAUGA X GAA AUGCUAUA
2499	AUAGCAUUU AUGAGGAG	CUCCUCAU CUGAUGA X GAA AAUGCUAU
2500	UAGCAUUUA UGAGGAGA	UCUCCUCA CUGAUGA X GAA AAAUGCUA
2510	GAGGAGAUU UUGUUGUC	GACAACAA CUGAUGA X GAA AUCUCCUC
2511	AGGAGAUUU UGUUGUCA	UGACAACA CUGAUGA X GAA AAUCUCCU
2512	GGAGAUUUU GUUGUCAG	CUGACAAC CUGAUGA X GAA AAAUCUCC
2515	GAUUUUGUU GUCAGCUU	AAGCUGAC CUGAUGA X GAA ACAAAAUC
2518	UUUGUUGUC AGCUUGCU	AGCAAGCU CUGAUGA X GAA ACAACAAA
2523	UGUCAGCUU GCUUGAAA	UUUCAAGC CUGAUGA X GAA AGCUGACA
2527	AGCUUGCUU GAAAGUUA	UAACUUUC CUGAUGA X GAA AGCAAGCU
2534	UUGAAAGUU AUUAUGUA	UACAUAAU CUGAUGA X GAA ACUUUCAA
2535	UGAAAGUUA UUAUGUAU	AUACAUAA CUGAUGA X GAA AACUUUCA
2537	AAAGUUAUU AUGUAUGA	UCAUACAU CUGAUGA X GAA AUAACUUU
2538	AAGUUAUUA UGUAUGAA	UUCAUACA CUGAUGA X GAA AAUAACUU
2542	UAUUAUGUA UGAAUAGU	ACUAUUCA CUGAUGA X GAA ACAUAAUA
2548	GUAUGAAUA GUUUUAUU	AAUAAAAC CUGAUGA X GAA AUUCAUAC
2551	UGAAUAGUU UUAUUGAA	UUCAAUAA CUGAUGA X GAA ACUAUUCA
2552	GAAUAGUUU UAUUGAAA	UUUCAAUA CUGAUGA X GAA AACUAUUC
2553	AAUAGUUUU AUUGAAAA	UUUUCAAU CUGAUGA X GAA AAACUAUU
2554	AUAGUUUUA UUGAAAAA	UUUUUCAA CUGAUGA X GAA AAAACUAU

nt. Position	Target Sequence	Ribozyme Sequence
2556	AGUUUUAUU GAAAAAAU	AUUUUUUC CUGAUGA X GAA AUAAAACU
2565	GAAAAAAUU AUAUUUUU	AAAAAUAU CUGAUGA X GAA AUUUUUUC
2566	AAAAAAUUA UAUUUUUA	UAAAAAUA CUGAUGA X GAA AAUUUUUU
2568	AAAAUUAUA UUUUUAUU	AAUAAAAA CUGAUGA X GAA AUAAUUUU
2570	AAUUAUAUU UUUAUUCA	UGAAUAAA CUGAUGA X GAA AUAUAAUU
2571	AUUAUAUUU UUAUUCAG	CUGAAUAA CUGAUGA X GAA AAUAUAAU
2572	UUAUAUUUU UAUUCAGU	ACUGAAUA CUGAUGA X GAA AAAUAUAA
2573	UAUAUUUUU AUUCAGUA	UACUGAAU CUGAUGA X GAA AAAAUAUA
2574	AUAUUUUUA UUCAGUAA	UUACUGAA CUGAUGA X GAA AAAAAUAU
2576	AUUUUUAUU CAGUAAUU	AAUUACUG CUGAUGA X GAA AUAAAAAU
2577	UUUUUAUUC AGUAAUUU	AAAUUACU CUGAUGA X GAA AAUAAAAA
2581	UAUUCAGUA AUUUAAUU	AAUUAAAU CUGAUGA X GAA ACUGAAUA
2584	UCAGUAAUU UAAUUUUG	CAAAAUUA CUGAUGA X GAA AUUACUGA
2585	CAGUAAUUU AAUUUUGU	ACAAAAUU CUGAUGA X GAA AAUUACUG
2586	AGUAAUUUA AUUUUGUA	UACAAAAU CUGAUGA X GAA AAAUUACU
2589	AAUUUAAUU UUGUAAAU	AUUUACAA CUGAUGA X GAA AUUAAAUU
2590	AUUUAAUUU UGUAAAUG	CAUUUACA CUGAUGA X GAA AAUUAAAU
2591	UUUAAUUUU GUAAAUGC	GCAUUUAC CUGAUGA X GAA AAAUUAAA
2594	AAUUUUGUA AAUGCCAA	UUGGCAUU CUGAUGA X GAA ACAAAAUU
2617	AAAUGUGUU CGCUGCUA	UAGCAGCG CUGAUGA X GAA ACACAUUU
2618	AAUGUGUUC GCUGCUAU	AUAGCAGC CUGAUGA X GAA AACACAUU
2625	UCGCUGCUA UGGUUUUA	UAAAACCA CUGAUGA X GAA AGCAGCGA
2630	GCUAUGGUU UUAGCCUA	UAGGCUAA CUGAUGA X GAA ACCAUAGC
2631	CUAUGGUUU UAGCCUAU	AUAGGCUA CUGAUGA X GAA AACCAUAG
2632	UAUGGUUUU AGCCUAUA	UAUAGGCU CUGAUGA X GAA AAACCAUA
2633	AUGGUUUUA GCCUAUAG	CUAUAGGC CUGAUGA X GAA AAAACCAU
2638	UUUAGCCUA UAGUCAUG	CAUGACUA CUGAUGA X GAA AGGCUAAA
2640	UAGCCUAUA GUCAUGCU	AGCAUGAC CUGAUGA X GAA AUAGGCUA
2643	CCUAUAGUC AUGCUGCU	AGCAGCAU CUGAUGA X GAA ACUAUAGG
2652	AUGCUGCUA GCUAGUGU	ACACUAGC CUGAUGA X GAA AGCAGCAU
2656	UGCUAGCUA GUGUCAGG	CCUGACAC CUGAUGA X GAA AGCUAGCA
2661	GCUAGUGUC AGGGGGCA	UGCCCCCU CUGAUGA X GAA ACACUAGC
2672	GGGGCAAUA GAGCUUAG	CUAAGCUC CUGAUGA X GAA AUUGCCCC
2678	AUAGAGCUU AGAUGGAA	UUCCAUCU CUGAUGA X GAA AGCUCUAU
2679	UAGAGCUUA GAUGGAAA	UUUCCAUC CUGAUGA X GAA AAGCUCUA
2703	AAGAGACUC GGUGUUAG	CUAACACC CUGAUGA X GAA AGUCUCUU

nt. Position	Target Sequence	Ribozyme Sequence
2709	CUCGGUGUU AGAUAACG	CGUUAUCU CUGAUGA X GAA ACACCGAG
2710	UCGGUGUUA GAUAACGG	CCGUUAUC CUGAUGA X GAA AACACCGA
2714	UGUUAGAUA ACGGACUA	UAGUCCGU CUGAUGA X GAA AUCUAACA
2722	AACGGACUA UGCACUAG	CUAGUGCA CUGAUGA X GAA AGUCCGUU
2729	UAUGCACUA GUAUUCCA	UGGAAUAC CUGAUGA X GAA AGUGCAUA
2732	GCACUAGUA UUCCAGAC	GUCUGGAA CUGAUGA X GAA ACUAGUGC
2734	ACUAGUAUU CCAGACUU	AAGUCUGG CUGAUGA X GAA AUACUAGU
2735	CUAGUAUUC CAGACUUU	AAAGUCUG CUGAUGA X GAA AAUACUAG
2742	UCCAGACUU UUUUAUUU	AAAUAAAA CUGAUGA X GAA AGUCUGGA
2743	CCAGACUUU UUUAUUUU	AAAAUAAA CUGAUGA X GAA AAGUCUGG
2744	CAGACUUUU UUAUUUUU	AAAAAUAA CUGAUGA X GAA AAAGUCUG
2745	AGACUUUUU UAUUUUUU	AAAAAAUA CUGAUGA X GAA AAAAGUCU
2746	GACUUUUUU AUUUUUUA	UAAAAAAU CUGAUGA X GAA AAAAAGUC
2747	ACUUUUUUA UUUUUUAU	AUAAAAAA CUGAUGA X GAA AAAAAAGU
2749	UUUUUUAUU UUUUAUAU	AUAUAAAA CUGAUGA X GAA AUAAAAAA
2750	UUUUUAUUU UUUAUAUA	UAUAUAAA CUGAUGA X GAA AAUAAAAA
2751	UUUUAUUUU UUAUAUAU	AUAUAUAA CUGAUGA X GAA AAAUAAAA
2752	UUUAUUUUU UAUAUAUA	UAUAUAUA CUGAUGA X GAA AAAAUAAA
2753	UUAUUUUUU AUAUAUAU	AUAUAUAU CUGAUGA X GAA AAAAAUAA
2754	UAUUUUUUA UAUAUAUG	CAUAUAUA CUGAUGA X GAA AAAAAAUA
2756	UUUUUUAUA UAUAUGUA	UACAUAUA CUGAUGA X GAA AUAAAAAA
2758	UUUUAUAUA UAUGUACC	GGUACAUA CUGAUGA X GAA AUAUAAAA
2760	UUAUAUAUA UGUACCUU	AAGGUACA CUGAUGA X GAA AUAUAUAA
2764	AUAUAUGUA CCUUUUCC	GGAAAAGG CUGAUGA X GAA ACAUAUAU
2768	AUGUACCUU UUCCUUUU	AAAAGGAA CUGAUGA X GAA AGGUACAU
2769	UGUACCUUU UCCUUUUG	CAAAAGGA CUGAUGA X GAA AAGGUACA
2770	GUACCUUUU CCUUUUGU	ACAAAAGG CUGAUGA X GAA AAAGGUAC
2771	UACCUUUUC CUUUUGUC	GACAAAAG CUGAUGA X GAA AAAAGGUA
2774	CUUUUCCUU UUGUCAAU	AUUGACAA CUGAUGA X GAA AGGAAAAG
2775	UUUUCCUUU UGUCAAUU	AAUUGACA CUGAUGA X GAA AAGGAAAA
2776	UUUCCUUUU GUCAAUUG	CAAUUGAC CUGAUGA X GAA AAAGGAAA

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20 3252). The length of stem II may be ≥ 2 base-pairs.

Table XVI: Mouse c-myb Hairpin ribozyme and target sequences

Position	RZ	Substrate
24	GCGAGGCG AGAA GGGGCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGCCCCG GCC CGCCUCGC
28	CAUGGCGA AGAA GGCCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCGGCCC GCC UCGCCAUG
122	AUUUGGGC AGAA GCCCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUGGGCU GCU GCCCAAAU
125	CAGAUUUG AGAA GCAGCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGCUGCU GCC CAAAUCUG
216	UUCCAGUC AGAA GUUCCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGGAACA GAC GACUGGAA
245	UCCGGUUG AGAA GAUAAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUUAUCU GCC CAACCGGA
258	CACUGUAC AGAA GUCCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCGGACA GAU GUACAGUG
529	CUCUGCCC AGAA GUUCCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGGAACA GAU GGGCAGAG
551	GUCCGGGC AGAA GCUUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAAAGCU GCU GCCCGGAC
554	UCCGUCCG AGAA GCAGCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGCUGCU GCC CGGACGGA
559	AUCAGUCC AGAA GGGCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCCCG GAC GGACUGAU
563	CAUUAUCA AGAA GUCCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCGGACG GAC UGAUAAUG
656	CCACUGGC AGAA GGCUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCAGCCA GAC GCCAGUGG
728	UUGGAGAG AGAA GAGAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAUCUCA GCU CUCUCCAA
746	UGACGGAG AGAA GGCCAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUGGCCA GUC CUCCGUCA
822	UGCAAUGC AGAA GGAUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUAUCCU GUC GCAUUGCA

857	CCGCAGCC AGAA GAGGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCCCUCA GCC GGCUGCGG
861	GCUGCCGC AGAA GGCUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCAGCCG GCU GCGGCAGC
941	CUGUUGAC AGAA GGAGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGCUCCU GAU GUCAACAG
1040	GAGGUCUG AGAA GGUCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGGACCA GAC CAGACCUC
1045	CCCAUGAG AGAA GGUCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAGACCA GAC CUCAUGGG
1068	AAACAGGA AGAA GGUGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGCACCU GUU UCCUGUUU
1075	UUCUCCCA AGAA GGAAAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUUUCCU GUU UGGGAGAA
1106	GAUCUGCA AGAA GAGAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAUCUCU GCC UGCAGAUC
1113	GAGCCGGG AGAA GCAGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCCUGCA GAU CCCGGCUC
1120	AGGUAGGG AGAA GGGAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAUCCCG GCU CCCUACCU
1226	AAUCUAUA AGAA GGAGUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CACUCCA GUU UAUAGAUU
1340	UUUUCACA AGAA GGUCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGACCA GAC UGUGAAAA
1449	AUUUCUUG AGAA GCAAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCUUGCA GCU CAAGAAAU
1468	CUUCAGGG AGAA GUAUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAAUACG GUC CCCUGAAG
1490	GGGAGGGG AGAA GAGGUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UACCUCA GAC CCCCUCCC
1542	CCAGAUUC AGAA GAUUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGAAUCG GAU GAAUCUGG
1648	GUGGUUUG AGAA GAAGAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUCUUCU GCU CAAACCAC
1672	GGUGCUCA AGAA GUUCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGAACA GCC UGAGCACC

1688	CCUGCGAG AGAA GUUGGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCCAACU GUU CUCGCAGG
1713	UUUGGGGC AGAA GCCACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGUGGCA GAU GCCCCAAA
1740	GUCAUUAA AGAA GAGCUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAGCUCU GUU UUAAUGAC
1880	AGGCCGUC AGAA GGUCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGGACCA GAU GACGGCCU
1887	GGACCGGA AGAA GUCAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAUGACG GCC UCCGGUCC
1894	CCGAGCCG AGAA GGAGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCCUCCG GUC CGGCUCGG
1899	UAUUUCCG AGAA GGACCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGGUCCG GCU CGGAAAUA
1926	AGAGUUCG AGAA GAGAAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUUCUCA GCU CGAACUCU
2048	ACAACAAA AGAA GGCUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGAGCCU GAU UUUGUUGU
2068	CUGCUCUC AGAA GUUGUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UACAACA GUU GAGAGCAG
2170	UUAGGUAA AGAA GUUAUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAUAACA GUC UUACCUAA
2225	UUUAAAAA AGAA GAUUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUAAUCA GAU UUUUUAAA
2276	AAAUACUG AGAA GUUGUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UACAACA GAU CAGUAUUU
2519	UUCAAGCA AGAA GACAAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUUGUCA GCU UGCUUGAA
2717	AGUGCAUA AGAA GUUAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAUAACG GAC UAUGCACU
2737	AUAAAAAA AGAA GGAAUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UAUUCCA GAC UUUUUUAU

Table XVII: Rat c-myb (Region A) Hammerhead Ribozyme and Target Sequences (282 bp; nt. 428 start; human c-myb numbering system)

nt. Position	Target Sequence	Ribozyme Sequence
467	CCUGAGCUC AUCAAAGG	CCUUUGAU CUGAUGA X GAA AGCUCAGG
470	GAGCUCAUC AAAGGUCC	GGACCUUU CUGAUGA X GAA AUGAGCUC
477	UCAAAGGUC CCUGGACC	GGUCCAGG CUGAUGA X GAA ACCUUUGA
498	AAGAAGAUC AAAGAGUG	CACUCUUU CUGAUGA X GAA AUCUUCUU
509	AGAGUGAUA GAGCUUGU	ACAAGCUC CUGAUGA X GAA AUCACUCU
515	AUAGAGCUU GUCCAGAA	UUCUGGAC CUGAUGA X GAA AGCUCUAU
518	GAGCUUGUC CAGAAAUA	UAUUUCUG CUGAUGA X GAA ACAAGCUC
526	CCAGAAAUA CGGUCCGA	UCGGACCG CUGAUGA X GAA AUUUCUGG
531	AAUACGGUC CGAAGCGC	GCGCUUCG CUGAUGA X GAA ACCGUAUU
544	GCGCUGGUC UGUUAUUG	CAAUAACA CUGAUGA X GAA ACCAGCGC
548	UGGUCUGUU AUUGCCAA	UUGGCAAU CUGAUGA X GAA ACAGACCA
549	GGUCUGUUA UUGCCAAG	CUUGGCAA CUGAUGA X GAA AACAGACC
551	UCUGUUAUU GCCAAGCA	UGCUUGGC CUGAUGA X GAA AUAACAGA
562	CAAGCACUU AAAAGGGA	UCCCUUUU CUGAUGA X GAA AGUGCUUG
563	AAGCACUUA AAAGGGAG	CUCCCUUU CUGAUGA X GAA AAGUGCUU
575	GGGAGAAUU GGAAAACA	UGUUUUCC CUGAUGA X GAA AUUCUCCC
588	AACAAUGUC GGGAGAGG	CCUCUCCC CUGAUGA X GAA ACAUUGUU
609	ACAACCAUU UGAAUCCA	UGGAUUCA CUGAUGA X GAA AUGGUUGU
610	CAACCAUUU GAAUCCAG	CUGGAUUC CUGAUGA X GAA AAUGGUUG
615	AUUUGAAUC CAGAAGUU	AACUUCUG CUGAUGA X GAA AUUCAAAU
623	CCAGAAGUU AAGAAAAC	GUUUUCUU CUGAUGA X GAA ACUUCUGG
624	CAGAAGUUA AGAAAACC	GGUUUUCU CUGAUGA X GAA AACUUCUG
634	GAAAACCUC AUGGACAG	CUGUCCAU CUGAUGA X GAA AGGUUUUC
659	GACAGAAUC AUUUAUCA	UGAUAAAU CUGAUGA X GAA AUUCUGUC
662	AGAAUCAUU UAUCAGGC	GCCUGAUA CUGAUGA X GAA AUGAUUCU
663	GAAUCAUUU AUCAGGCA	UGCCUGAU CUGAUGA X GAA AAUGAUUC
664	AAUCAUUUA UCAGGCAC	GUGCCUGA CUGAUGA X GAA AAAUGAUU
666	UCAUUUAUC AGGCACAC	GUGUGCCU CUGAUGA X GAA AUAAAUGA

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20 3252). The length of stem II may be ≥ 2 base-pairs.

Table XVIII: Rat c-myb (Region B) Hammerhead Ribozyme and Target Sequences (262 bp; nt. 1421 start; human c-myb numbering system)

nt. Position	Target Sequence	Ribozyme Sequence
1429	CUCGGGCUU AGAUACGC	GCGUAUCU CUGAUGA X GAA AGCCCGAG
1430	UCGGGCUUA GAUACGCC	GGCGUAUC CUGAUGA X GAA AAGCCCGA
1434	GCUUAGAUA CGCCUACU	AGUAGGCG CUGAUGA X GAA AUCUAAGC
1440	AUACGCCUA CUUUACCC	GGGUAAAG CUGAUGA X GAA AGGCGUAU
1443	CGCCUACUU UACCCUCC	GGAGGGUA CUGAUGA X GAA AGUAGGCG
1444	GCCUACUUU ACCCUCCA	UGGAGGGU CUGAUGA X GAA AAGUAGGC
1445	CCUACUUUA CCCUCCAC	GUGGAGGG CUGAUGA X GAA AAAGUAGG
1450	UUUACCCUC CACGCCUC	GAGGCGUG CUGAUGA X GAA AGGGUAAA
1458	CCACGCCUC UCAUUGGU	ACCAAUGA CUGAUGA X GAA AGGCGUGG
1460	ACGCCUCUC AUUGGUCA	UGACCAAU CUGAUGA X GAA AGAGGCGU
1463	CCUCUCAUU GGUCACAA	UUGUGACC CUGAUGA X GAA AUGAGAGG
1467	UCAUUGGUC ACAAACUG	CAGUUUGU CUGAUGA X GAA ACCAAUGA
1485	CACCGUGUC ACCGAGAC	GUCUCGGU CUGAUGA X GAA ACACGGUG
1509	UGAAAACUN AAAAGGAA	UUCCUUUU CUGAUGA X GAA AGUUUUCA
1522	GGAAAACUC NAUCUUUA	UAAAGAUN CUGAUGA X GAA AGUUUUCC
1526	AACUCNAUC UUUAGAAC	GUUCUAAA CUGAUGA X GAA AUNGAGUU
1528	CUCNAUCUU UAGAACUC	GAGUUCUA CUGAUGA X GAA AGAUNGAG
1529	UCNAUCUUU AGAACUCC	GGAGUUCU CUGAUGA X GAA AAGAUNGA
1530	CNAUCUUUA GAACUCCA	UGGAGUUC CUGAUGA X GAA AAAGAUNG
1536	UUAGAACUC CAGCUAUC	GAUAGCUG CUGAUGA X GAA AGUUCUAA
1542	CUCCAGCUA UCAAAAGG	CCUUUUGA CUGAUGA X GAA AGCUGGAG
1544	CCAGCUAUC AAAAGGUN	NACCUUUU CUGAUGA X GAA AUAGCUGG
1552	CAAAAGGUN AAUCCUCG	CGAGGAUU CUGAUGA X GAA ACCUUUUG
1556	AGGUNAAUC CUCGAAAG	CUUUCGAG CUGAUGA X GAA AUUNACCU
1559	UNAAUCCUC GAAAGCUC	GAGCUUUC CUGAUGA X GAA AGGAUUNA
1567	CGAAAGCUC UCCCAGAA	UUCUGGGA CUGAUGA X GAA AGCUUUCG
1569	AAAGCUCUC CCAGAACU	AGUUCUGG CUGAUGA X GAA AGAGCUUU
1578	CCAGAACUC CCACACCA	UGGUGUGG CUGAUGA X GAA AGUUCUGG
1588	CACACCAUU CAAACAUG	CAUGUUUG CUGAUGA X GAA AUGGUGUG
1589	ACACCAUUC AAACAUGC	GCAUGUUU CUGAUGA X GAA AAUGGUGU
1608	UGGCAGCUC AAGAAAUU	AAUUUCUU CUGAUGA X GAA AGCUGCCA
1616	CAAGAAAUU AAAUACGG	CCGUAUUU CUGAUGA X GAA AUUUCUUG

nt. Position	Target Sequence	Ribozyme Sequence
1429	CUCGGGCUU AGAUACGC	GCGUAUCU CUGAUGA X GAA AGCCCGAG
1617	AAGAAAUUA AAUACGGU	ACCGUAUU CUGAUGA X GAA AAUUUCUU
1621	AAUUAAAUA CGGUCCCC	GGGGACCG CUGAUGA X GAA AUUUAAUU
1626	AAUACGGUC CCCUGAAG	CUUCAGGG CUGAUGA X GAA ACCGUAUU
1640	AAGAUGCUA CCUNAGAC	GUCUNAGG CUGAUGA X GAA AGCAUCUU
1644	UGCUACCUN AGACCCCC	GGGGGUCU CUGAUGA X GAA AGGUAGCA
1654	GACCCCCUN UNAUGUAG	CUACAUNA CUGAUGA X GAA AGGGGGUC
1656	CCCCCUNUN AUGUAGUN	NACUACAU CUGAUGA X GAA ANAGGGGG
1661	UNUNAUGUA GUNNNANA	UNUNNNAC CUGAUGA X GAA ACAUNANA
1664	NAUGUAGUN NNANACCU	AGGUNUNN CUGAUGA X GAA ACUACAUN
1673	NNANACCUN CANGAUGU	ACAUCNUG CUGAUGA X GAA AGGUNUNN

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20 3252). The length of stem II may be ≥ 2 base-pairs.

Table XIX: Rat c-myb (Region A) Hairpin Ribozyme and Target Sequences (282 bp; nt. 428 start; human numbering system)

Position	RZ	Substrate
528	GCGCUUCG AGAA GUAUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAAUACG GUC CGAAGCGC
690	UUCUGCCC AGAA GUUUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGAAACA GAU GGGCAGAA

Table XX: Rat c-myb (Region B) Hairpin Ribozyme and Target Sequences (262 bp; nt. 1421 start; human numbering system)

Posi-tion	RZ	Substrate
1495	UUUUCACA AGAA GGUCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGACCA GAC UGUGAAAA
1604	AUUUCUUG AGAA GCCAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCUGGCA GCU CAAGAAAU
1623	CUUCAGGG AGAA GUAUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAAUACG GUC CCCUGAAG

Table XXI: Porcine c-myb (Region A) Hammerhead Ribozyme and Target Sequence (266 bp; nt. 458 start; human c-myb numbering system)

nt. Posi-tion	Target Sequence	Ribozyme Sequence
467	CCUNAUCUC AUCAAGGG	CCCUUGAU CUGAUGA X GAA AGAUNAGG
470	NAUCUCAUC AAGGGUCC	GGACCCUU CUGAUGA X GAA AUGAGAUN
477	UCAAGGGUC CUUGGACC	GGUCCAAG CUGAUGA X GAA ACCCUUGA
480	AGGGUCCUU GGACCAAA	UUUGGUCC CUGAUGA X GAA AGGACCCU
498	AAGAAGAUC AGAGAGUG	CACUCUCU CUGAUGA X GAA AUCUUCUU
509	AGAGUGAUA GAGCUUGU	ACAAGCUC CUGAUGA X GAA AUCACUCU
515	AUAGAGCUU GUACAGAA	UUCUGUAC CUGAUGA X GAA AGCUCUAU
518	GAGCUUGUA CAGAAAUA	UAUUUCUG CUGAUGA X GAA ACAAGCUC
526	ACAGAAAUA CGGUCCGA	UCGGACCG CUGAUGA X GAA AUUUCUGU
531	AAUACGGUC CGAAACGU	ACGUUUCG CUGAUGA X GAA ACCGUAUU
540	CGAAACGUU GGUCUGUU	AACAGACC CUGAUGA X GAA ACGUUUCG
544	ACGUUGGUC UGUUAUUG	CAAUAACA CUGAUGA X GAA ACCAACGU
548	UGGUCUGUU AUUGCCAA	UUGGCAAU CUGAUGA X GAA ACAGACCA
549	GGUCUGUUA UUGCCAAG	CUUGGCAA CUGAUGA X GAA AACAGACC
551	UCUGUUAUU GCCAAGCA	UGCUUGGC CUGAUGA X GAA AUAACAGA
562	CAAGCACUU AAAGGGGA	UCCCCUUU CUGAUGA X GAA AGUGCUUG
563	AAGCACUUA AAGGGGAG	CUCCCCUU CUGAUGA X GAA AAGUGCUU
575	GGGAGAAUU GGAAAACA	UGUUUUCC CUGAUGA X GAA AUUCUCCC
588	AACAAUGUA GGGAGAGG	CCUCUCCC CUGAUGA X GAA ACAUUGUU
603	GGUGGCAUA ACCACUUG	CAAGUGGU CUGAUGA X GAA AUGCCACC
610	UAACCACUU GAAUCCAG	CUGGAUUC CUGAUGA X GAA AGUGGUUA

nt. Position	Target Sequence	Ribozyme Sequence
615	ACUUGAAUC CAGAAGUU	AACUUCUG CUGAUGA X GAA AUUCAAGU
623	CCAGAAGUU AAGAAAAC	GUUUUCUU CUGAUGA X GAA ACUUCUGG
624	CAGAAGUUA AGAAAACC	GGUUUUCU CUGAUGA X GAA AACUUCUG
634	GAAAACCUC CUGGACAG	CUGUCCAG CUGAUGA X GAA AGGUUUUC
659	GACAGAAUU AUUUACCA	UGGUAAAU CUGAUGA X GAA AUUCUGUC
660	ACAGAAUUA UUUACCAG	CUGGUAAA CUGAUGA X GAA AAUUCUGU
662	AGAAUUAUU UACCAGGC	GCCUGGUA CUGAUGA X GAA AUAAUUCU
663	GAAUUAUUU ACCAGGCA	UGCCUGGU CUGAUGA X GAA AAUAAUUC
664	AAUUAUUUA CCAGGCAC	GUGCCUGG CUGAUGA X GAA AAAUAAUU
704	GCGGAAAUC GCAAAGCU	AGCUUUGC CUGAUGA X GAA AUUUCCGC
713	GCAAAGCUA CUGCCUGG	CCAGGCAG CUGAUGA X GAA AGCUUUGC

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20 3252). The length of stem II may be ≥ 2 base-pairs.

Table XXII: Porcine c-myb (Region B) Hammerhead Ribozyme and Target Sequence (308 bp; nt. 1386 start; human c-myb numbering system)

nt. Position	Target Sequence	Ribozyme Sequence
1394	GAUUCUUUC UUAAACAC	GUGUUUAA CUGAUGA X GAA AAAGAAUC
1396	UUCUUUCUU AAACACUU	AAGUGUUU CUGAUGA X GAA AGAAAGAA
1397	UCUUUCUUA AACACUUC	GAAGUGUU CUGAUGA X GAA AAGAAAGA
1404	UAAACACUU CCAAUAAC	GUUAUUGG CUGAUGA X GAA AGUGUUUA
1405	AAACACUUC CAAUAACC	GGUUAUUG CUGAUGA X GAA AAGUGUUU
1410	CUUCCAAUA ACCAUGAA	UUCAUGGU CUGAUGA X GAA AUUGGAAG
1423	UGAAAACUU AGACUUGG	CCAAGUCU CUGAUGA X GAA AGUUUUCA
1424	GAAAACUUA GACUUGGA	UCCAAGUC CUGAUGA X GAA AAGUUUUC
1429	CUUAGACUU GGAAAUGC	GCAUUUCC CUGAUGA X GAA AGUCUAAG
1440	AAAUGCCUU CUUUAACG	CGUUAAAG CUGAUGA X GAA AGGCAUUU
1441	AAUGCCUUC UUUAACGU	ACGUUAAA CUGAUGA X GAA AAGGCAUU
1443	UGCCUUCUU UAACGUCC	GGACGUUA CUGAUGA X GAA AGAAGGCA
1444	GCCUUCUUU AACGUCCA	UGGACGUU CUGAUGA X GAA AAGAAGGC
1445	CCUUCUUUA ACGUCCAC	GUGGACGU CUGAUGA X GAA AAAGAAGG

1450	UUUAACGUC CACGCCUC	GAGGCGUG CUGAUGA X GAA ACGUUAAA
1458	CCACGCCUC UCAGUGGU	ACCACUGA CUGAUGA X GAA AGGCGUGG
1460	ACGCCUCUC AGUGGUCA	UGACCACU CUGAUGA X GAA AGAGGCGU
1467	UCAGUGGUC ACAAAUUG	CAAUUUGU CUGAUGA X GAA ACCACUGA
1474	UCACAAAUU GACUGUUA	UAACAGUC CUGAUGA X GAA AUUUGUGA
1481	UUGACUGUU ACAACACC	GGUGUUGU CUGAUGA X GAA ACAGUCAA
1482	UGACUGUUA CAACACCA	UGGUGUUG CUGAUGA X GAA AACAGUCA
1492	AACACCAUU UCAUAGAG	CUCUAUGA CUGAUGA X GAA AUGGUGUU
1493	ACACCAUUU CAUAGAGA	UCUCUAUG CUGAUGA X GAA AAUGGUGU
1494	CACCAUUUC AUAGAGAC	GUCUCUAU CUGAUGA X GAA AAAUGGUG
1497	CAUUUCAUA GAGACCAG	CUGGUCUC CUGAUGA X GAA AUGAAAUG
1530	AGGAAAAUA CAUAUUUU	AAAAUAUG CUGAUGA X GAA AUUUUCCU
1534	AAAUACAUA UUUUUGAA	UUCAAAAA CUGAUGA X GAA AUGUAUUU
1536	AUACAUAUU UUUGAACU	AGUUCAAA CUGAUGA X GAA AUAUGUAU
1537	UACAUAUUU UUGAACUC	GAGUUCAA CUGAUGA X GAA AAUAUGUA
1538	ACAUAUUUU UGAACUCC	GGAGUUCA CUGAUGA X GAA AAAUAUGU
1539	CAUAUUUUU GAACUCCG	CGGAGUUC CUGAUGA X GAA AAAAUAUG
1545	UUUGAACUC CGGCUAUC	GAUAGCCG CUGAUGA X GAA AGUUCAAA
1551	CUCCGGCUA UCAAAAGG	CCUUUUGA CUGAUGA X GAA AGCCGGAG
1553	CCGGCUAUC AAAAGGUC	GACCUUUU CUGAUGA X GAA AUAGCCGG
1561	CAAAAGGUC AAUCCUGG	CCAGGAUU CUGAUGA X GAA ACCUUUUG
1565	AGGUCAAUC CUGGAAAG	CUUUCCAG CUGAUGA X GAA AUUGACCU
1576	GGAAAGCUC UCCAAGAA	UUCUUGGA CUGAUGA X GAA AGCUUUCC
1578	AAAGCUCUC CAAGAACU	AGUUCUUG CUGAUGA X GAA AGAGCUUU
1587	CAAGAACUC CUACACCG	CGGUGUAG CUGAUGA X GAA AGUUCUUG
1590	GAACUCCUA CACCGUUC	GAACGGUG CUGAUGA X GAA AGGAGUUC
1597	UACACCGUU CAAACAUG	CAUGUUUG CUGAUGA X GAA ACGGUGUA
1598	ACACCGUUC AAACAUGC	GCAUGUUU CUGAUGA X GAA AACGGUGU
1610	CAUGCACUC GCAGCUCA	UGAGCUGC CUGAUGA X GAA AGUGCAUG
1617	UCGCAGCUC AAGAAAUU	AAUUUCUU CUGAUGA X GAA AGCUGCGA
1625	CAAGAAAUU AAAUAUGG	CCAUAUUU CUGAUGA X GAA AUUUCUUG
1626	AAGAAAUUA AAUAUGGU	ACCAUAUU CUGAUGA X GAA AAUUUCUU
1630	AAUUAAAUA UGGUCCCC	GGGGACCA CUGAUGA X GAA AUUUAAUU
1635	AAUAUGGUC CCCUGAAG	CUUCAGGG CUGAUGA X GAA ACCAUAUU
1649	AAGAUGCUA CCUCAGAC	GUCUGAGG CUGAUGA X GAA AGCAUCUU
1653	UGCUACCUC AGACACCA	UGGUGUCU CUGAUGA X GAA AGGUAGCA
1663	GACACCAUC UCAUUUAG	CUAAAUGA CUGAUGA X GAA AUGGUGUC
1665	CACCAUCUC AUUUAGUA	UACUAAAU CUGAUGA X GAA AGAUGGUG
1668	CAUCUCAUU UAGUAGAA	UUCUACUA CUGAUGA X GAA AUGAGAUG

1669	AUCUCAUUU AGUAGAAG	CUUCUACU	CUGAUGA	X	GAA	AAUGAGAU
1670	UCUCAUUUA GUAGAAGA	UCUUCUAC	CUGAUGA	X	GAA	AAAUGAGA
1673	CAUUUAGUA GAAGACCU	AGGUCUUC	CUGAUGA	X	GAA	ACUAAAUG

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20 3252). The length of stem II may be ≥ 2 base-pairs.

Table XXIII: Porcine c-myb (region A) Hairpin Ribozyme and Target Sequence (266bp; nt. 458 start; Human numbering system)

Posi-tion	RZ	Substrate
528	ACGUUUCG AGAA GUAUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAAUACG GUC CGAAACGU
690	UUCCGCCC AGAA GUUCCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGGAACA GAU GGGCGGAA

Table XXIV: Porcine c-myb (region B) Hairpin Ribozyme and Target Sequence (308 bp; nt. 1386 start; Human numbering system)

Posi-tion	Hairpin Ribozyme	Substrate
1504	UUUUCACA AGAA GGUCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGACCA GAC UGUGAAAA
1594	CAUGUUUG AGAA GUGUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUACACC GUU CAAACAUG
1613	AUUUCUUG AGAA GCGAGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACUCGCA GCU CAAGAAAU

Claims

1. An enzymatic nucleic acid molecule which cleaves *c-myb* RNA, wherein the the binding arms of said nucleic acid contain sequences complementary to the sequences defined in Tables II, XII-XXIV.

2. An enzymatic nucleic acid molecule which cleaves RNA produced from a gene selected from one encoding *c-fos*, *oct-1*, SRF, PDGF receptor, bFGF receptor, angiotensin II, and endothelium-derived relaxing factor.

3. The enzymatic nucleic acid molecule of claims 1 or 2 wherein said nucleic acid molecule is in a hammerhead motif.

4. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said nucleic acid molecule is in a hairpin, hepatitis delta virus, VS nucleic acid, group I intron, or RNAseP nucleic acid motif.

5. The enzymatic nucleic acid molecule of claim 3 or 4, wherein said nucleic acid comprises between 12 and 100 bases complementary to said mRNA.

6. The enzymatic nucleic acid molecule of claim 5, wherein said nucleic acid comprises between 14 and 24 bases complementary to said mRNA.

7. Enzymatic nucleic acid molecule consisting essentially of any sequence selected from the group of sequences listed in Tables III, XII-XXIV.

8. A mammalian cell including an enzymatic nucleic acid molecule of any one of claims 1 or 2.

9. The cell of claim 8, wherein said cell is a human cell.

10. An expression vector including nucleic acid encoding an enzymatic nucleic acid molecule or multiple enzymatic molecules of claims 1 or 2 in a manner which allows expression of that enzymatic RNA molecule(s) within a mammalian cell.

11. A mammalian cell including an expression vector of claim 10.

12. The cell of claim 13, wherein said cell is a human cell.

13. A method for treatment of a stenotic condition by administering to a patient an enzymatic nucleic acid molecule of claims 1 or 2, or an enzymatic nucleic acid molecule which cleaves RNA produced from the gene c-myb.

14. A method for treatment of a stenotic condition by administering to a patient an expression vector of claim 10.

15. The method of claims 13 or 14, wherein said patient is a human.

16. A method for treatment of cancer by administering to a patient or a patient's cells an enzymatic nucleic acid molecule of claims 1 or 2.

17. A method for treatment of cancer by administering to a patient or a patient's cells an expression vector of claim 10.

18. The method of claims 16 or 17, wherein said patient is a human.

19. Method for administration of an enzymatic nucleic acid by mixing said nucleic acid with a chemical

selected from the group consisting of chloroquine, ammonium chloride, carbonyl cyanide *p*-trifluoromethoxy phenyl hydrazone (FCCP), monensin, colchicine, amphipathic peptides, viral proteins, and viral particles.

20. The enzymatic nucleic acid of claim 3, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises phosphorothioate linkages at at least three of the six 5' terminal nucleotides, and wherein said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of said nucleic acid, and wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'- end modification.

21. The enzymatic nucleic acid of claim 20, wherein said nucleic acid comprises a 3'-3' linked inverted ribose moeity at said 3' end.

22. The enzymatic nucleic acid of claim 3, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises of phosphorothioate linkages at at least three of the six 5' terminal nucleotides, and wherein said nucleic acid comprises a 2'-amino modification at position No. 4 and/or at position No. 7 of said nucleic acid, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moeity at its 3' end.

23. The enzymatic nucleic acid of claim 3, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises phosphorothioate linkages at at least three of the six 5' terminal nucleotides, and wherein said nucleic acid comprises non-nucleotide substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule,

wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moeity at its 3' end.

24. The enzymatic nucleic acid of claim 3, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises phosphorothioate linkages at at least three of the six 5' terminal nucleotides, and wherein said nucleic acid comprises 6-methyl uridine substitutions at position No. 4 and/or at position No. 7 of the said nucleic acid molecule, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moeity at its 3' end.

25. The enzymatic nucleic acid of claim 3, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises phosphorothioate linkages at at least three of thje six 5' terminal nucleotides, wherein said nucleic acid comprises 2'-C-allyl modification at position No. 4 of the said nucleic acid, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 2'-3' linked inverted ribose or thymidine moeity at its 3' end.

26. Oligonucleotide having complementarity to c-myb at at least 5 contiguous bases comprising a 2'-5'-linked adenylate residue having a 5'-phosphate.

27. The oligonucleotide of claim 26, having enzymatic activity on c-myb RNA.

28. The oligonucleotide of claim 26, comprising at least 20 bases able to form a hybrid with c-myb RNA.

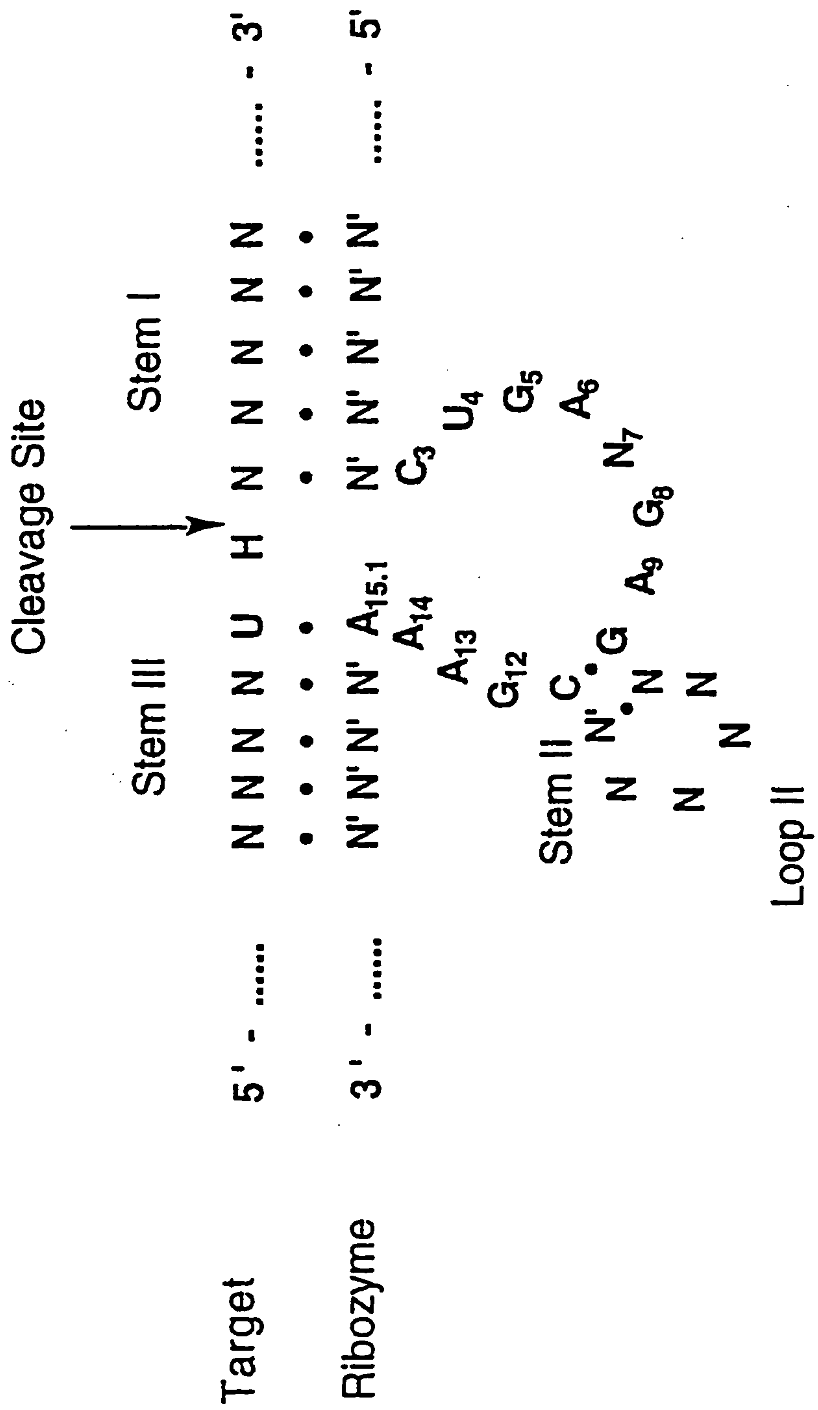
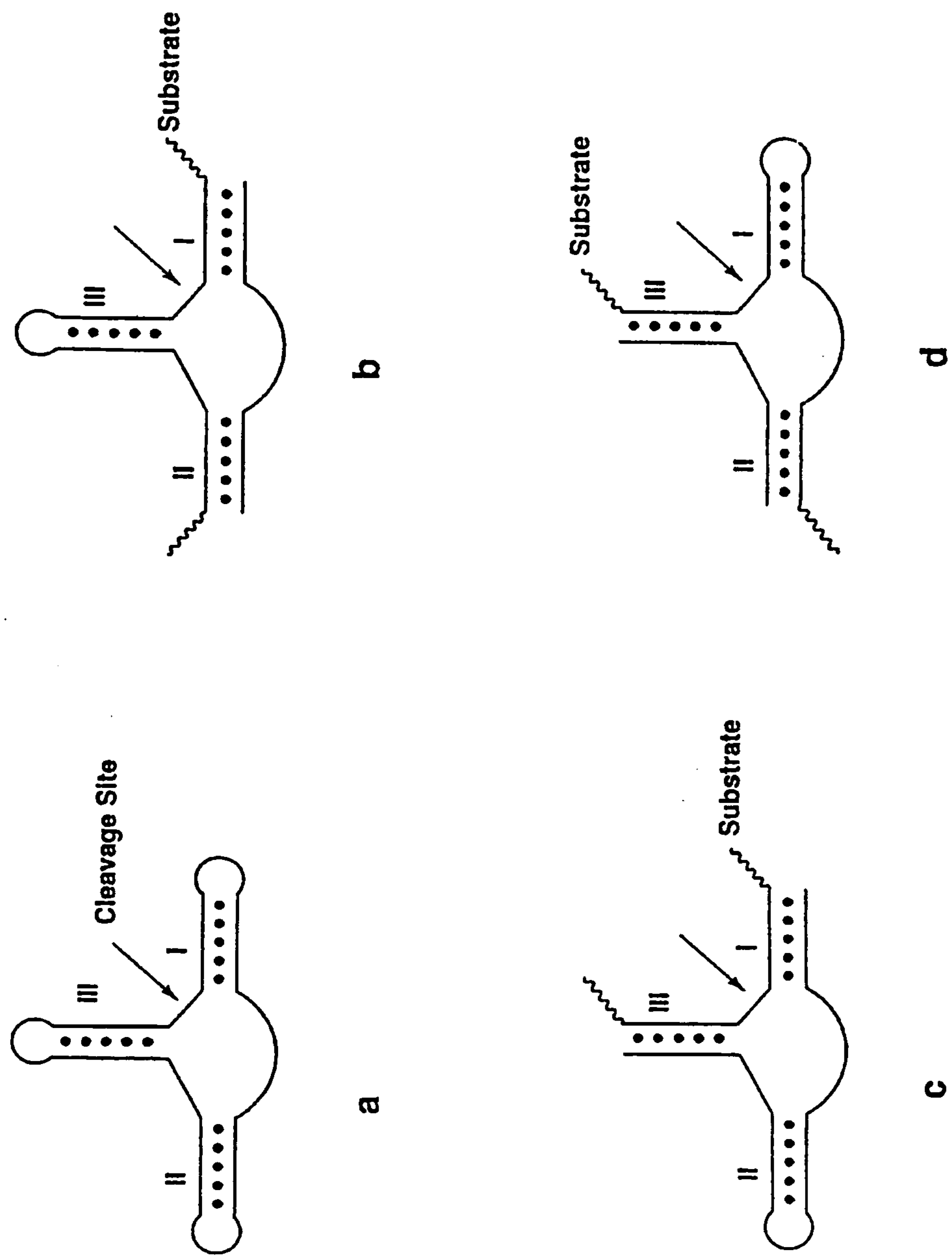

Target
Ribozyme
5' - ......
3' - ......
...... - 3'
...... - 5'
Cleavage Site
Stem I
Stem II
Stem III
Loop II

FIG. 1.

FIG. 2.


Cleavage Site
I
II
III
a
Substrate
I
II
III
b
Substrate
I
II
III
c
Substrate
I
II
III
d

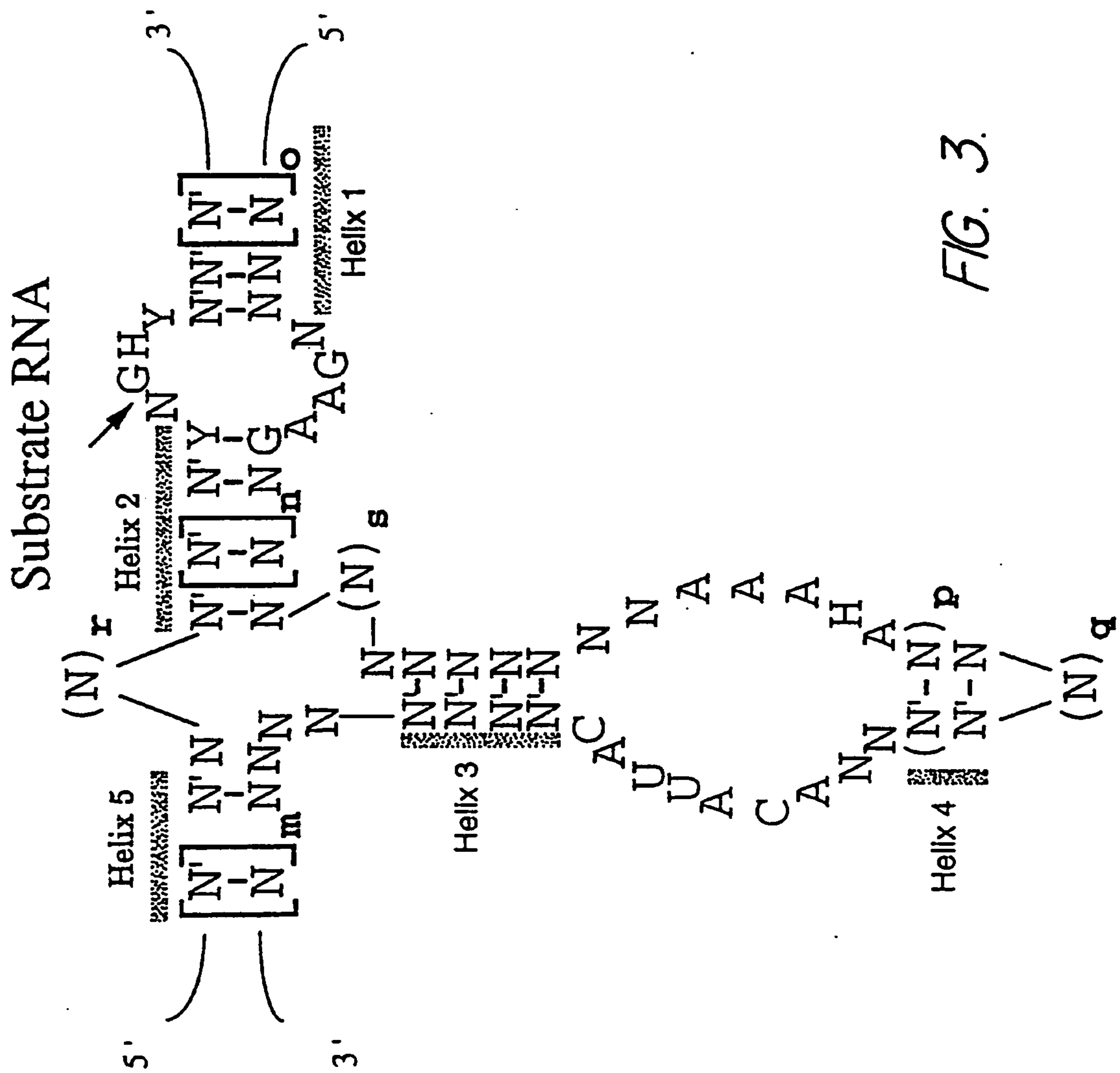


FIG. 3

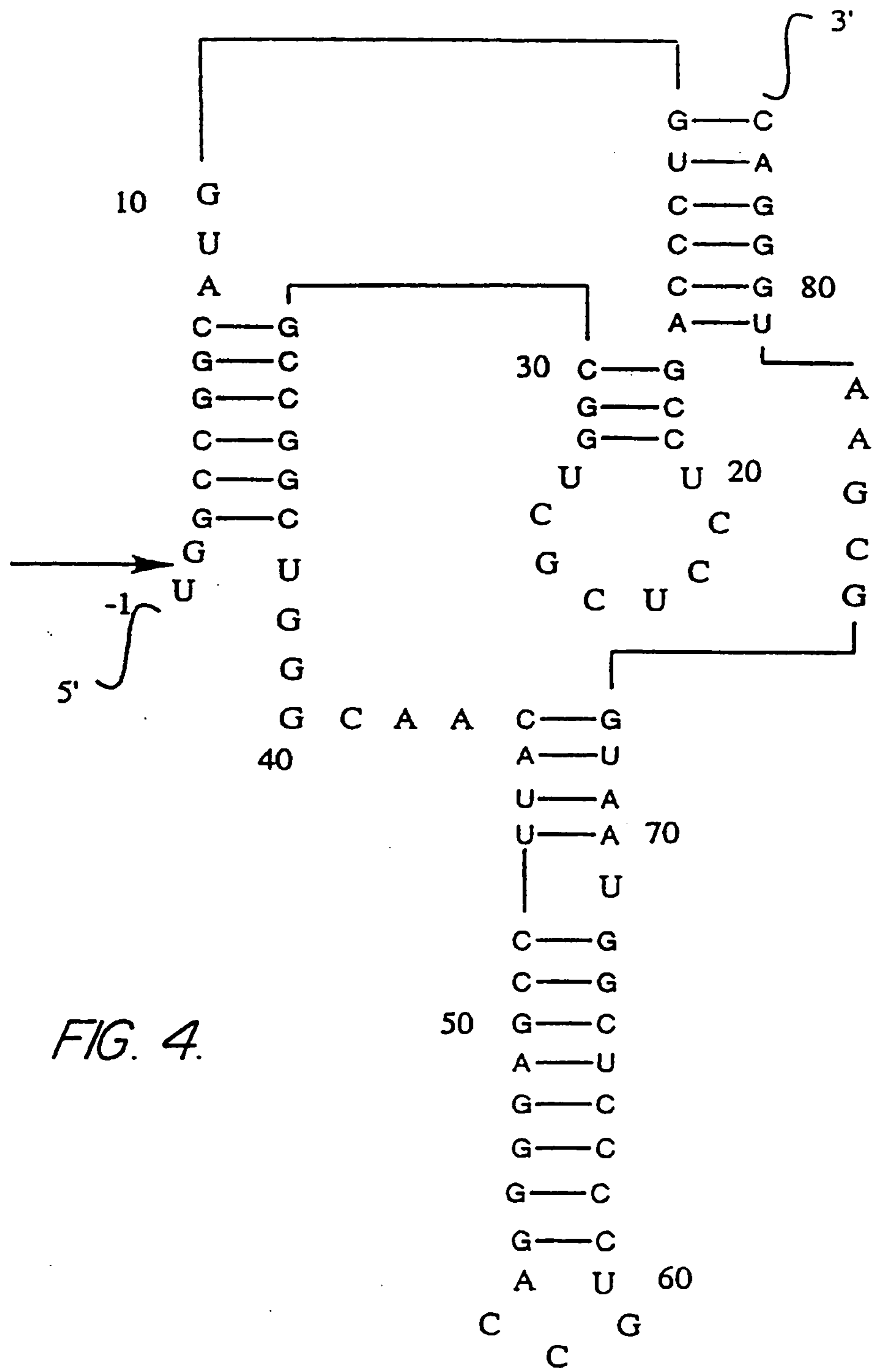


FIG. 4.

FIG. 5

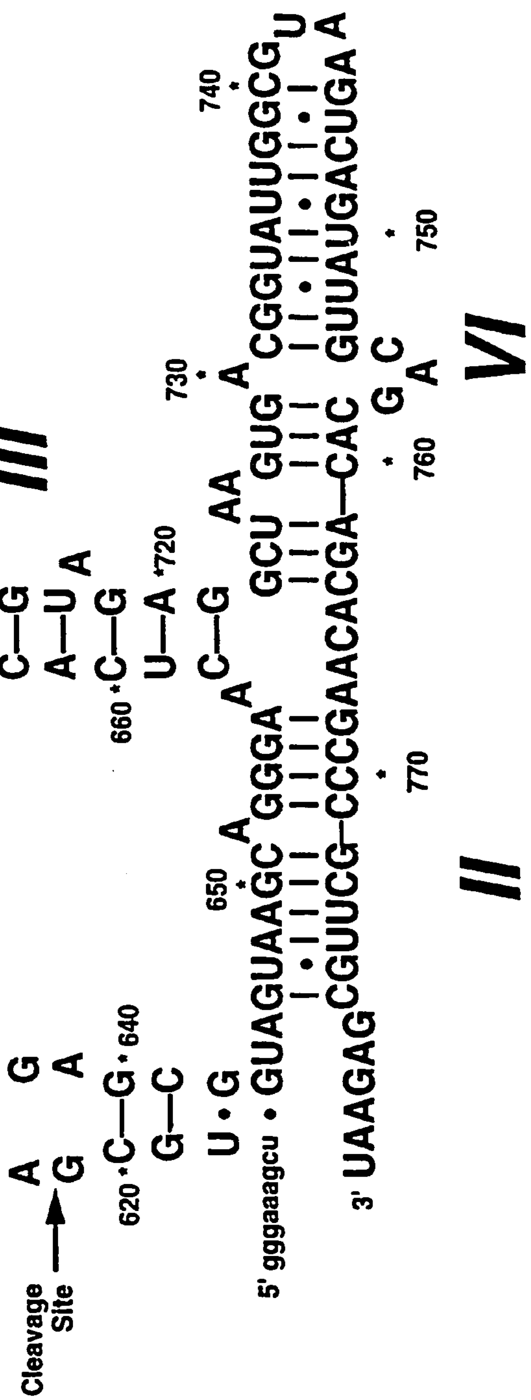
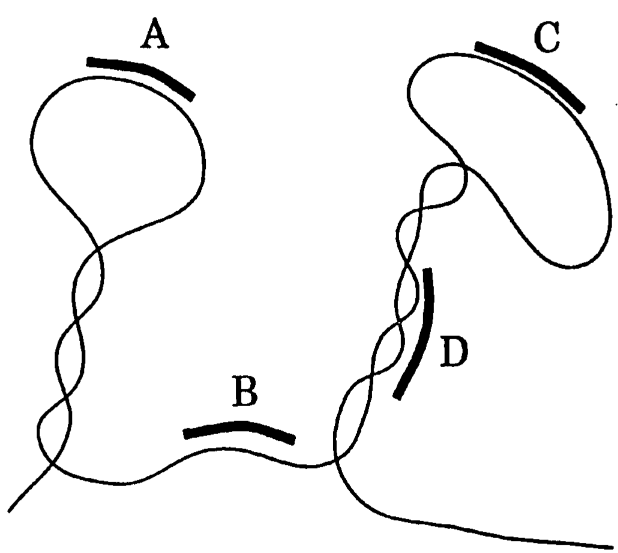
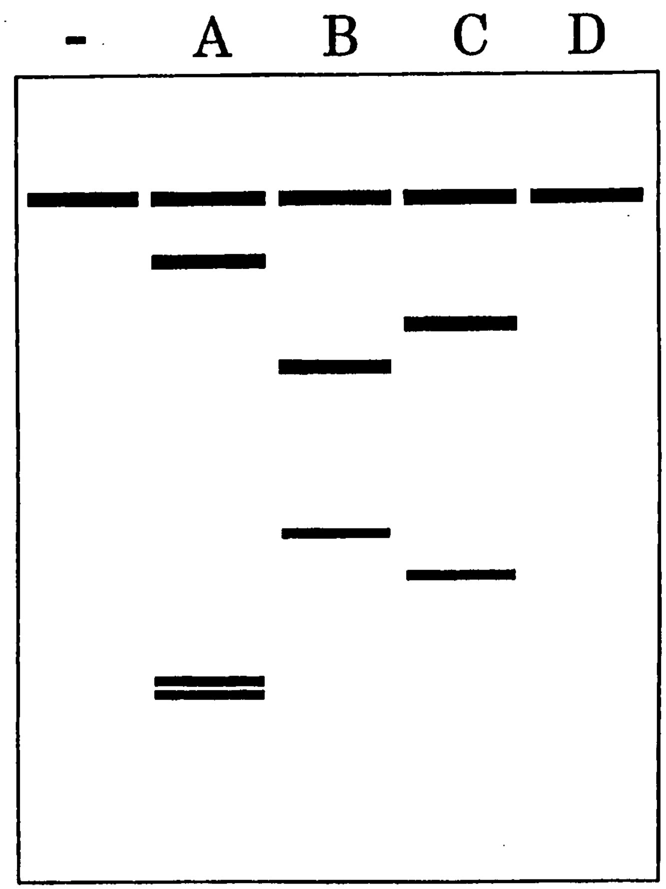

I
II
III
IV
V
VI
Cleavage Site
5' gggaaagcu
3' UAAGAG
620
630
640
650
660
670
680
690
700
720
730
740
750
760
770

FIG. 6.





- Body-labeled transcript
  (not purified)
- DNA oligo
  (10 nM, 100 nM and 1000 nM)
- RNAse H
  (0.08 -1.0 u/μl)
- $37^{o}C$, 10 min

FIG. 7.

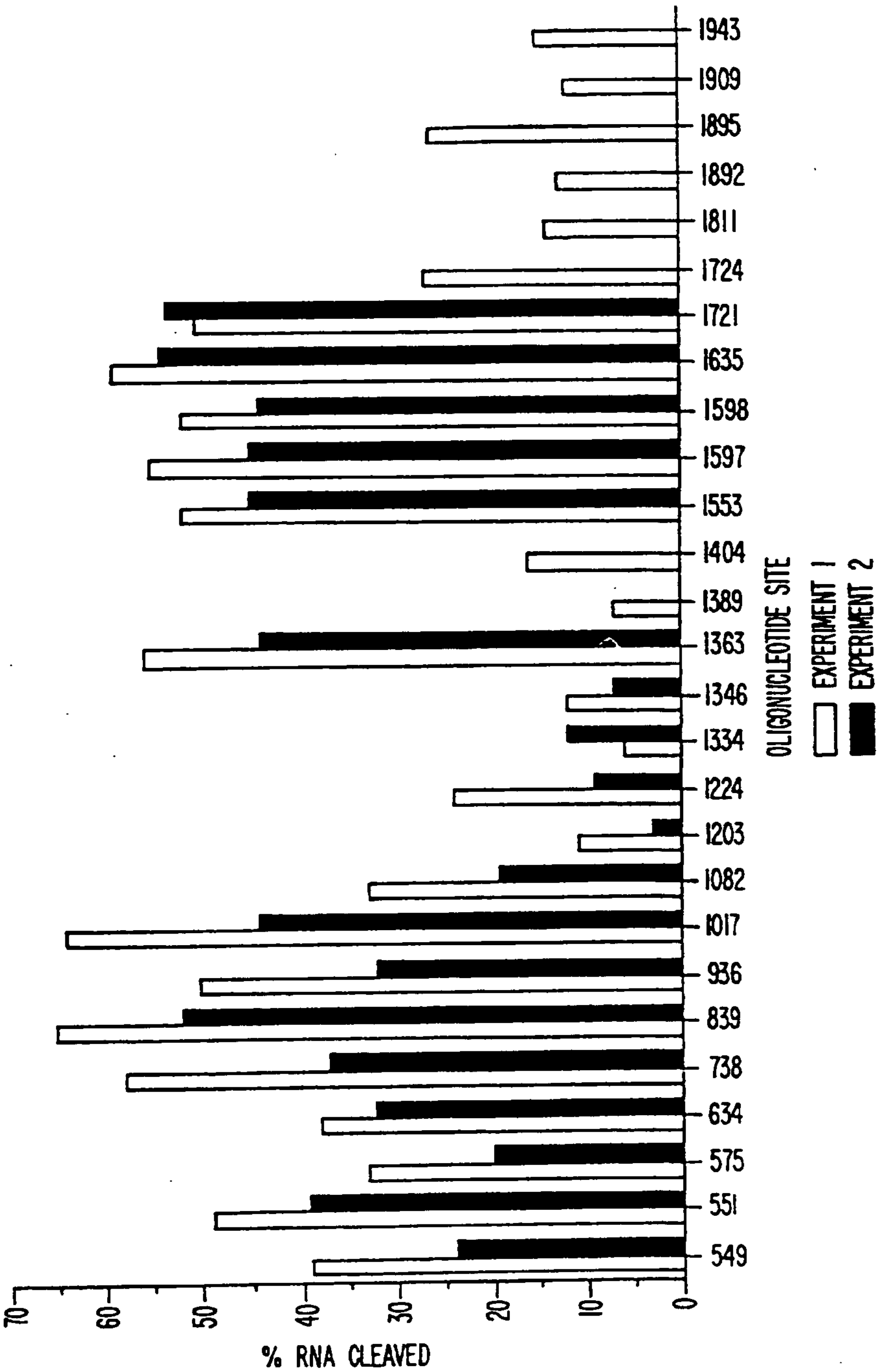
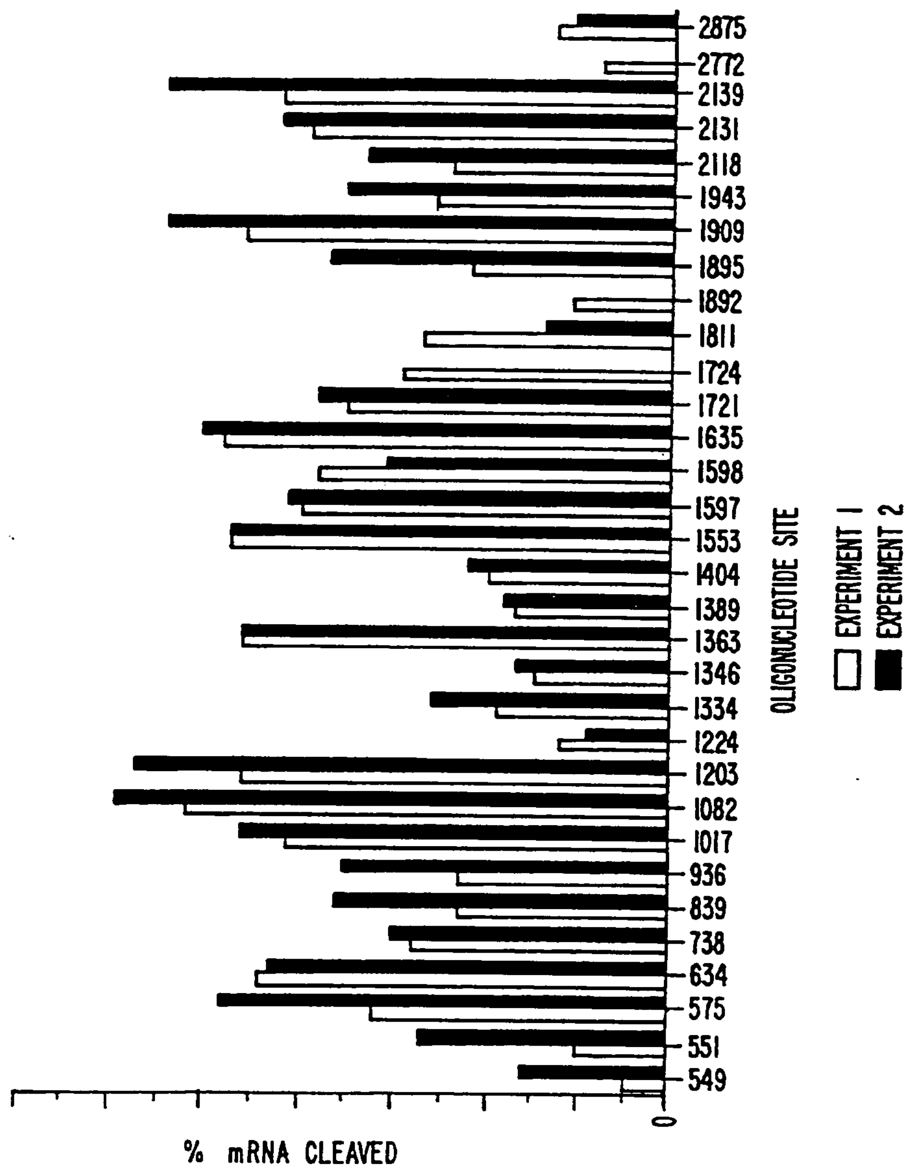
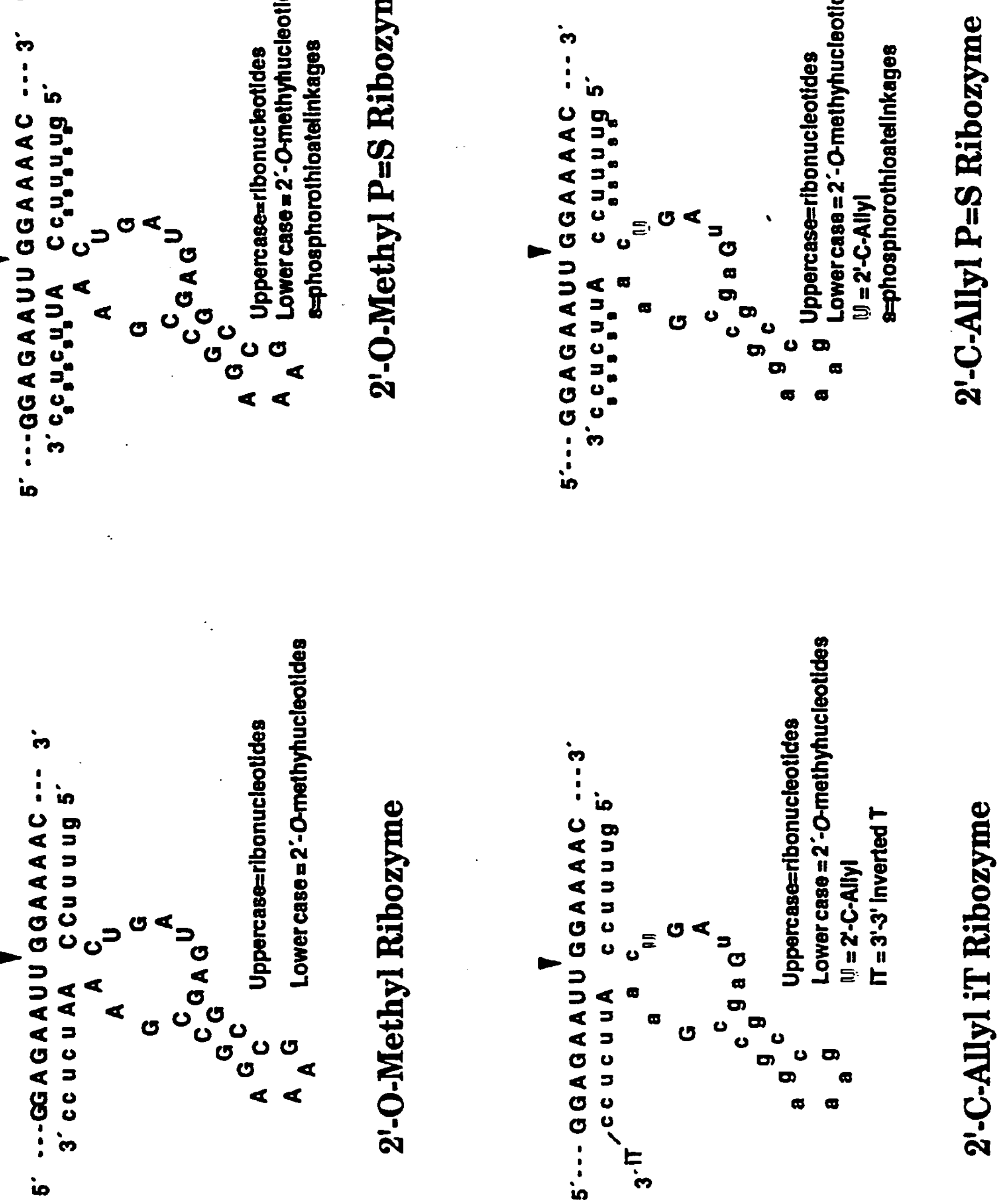

% RNA CLEAVED
0
10
20
30
40
50
60
70
549
551
575
634
738
839
936
1017
1082
1203
1224
1334
1346
1363
1389
1404
1553
1597
1598
1635
1721
1724
1811
1892
1895
1909
1943
OLIGONUCLEOTIDE SITE
EXPERIMENT 1
EXPERIMENT 2

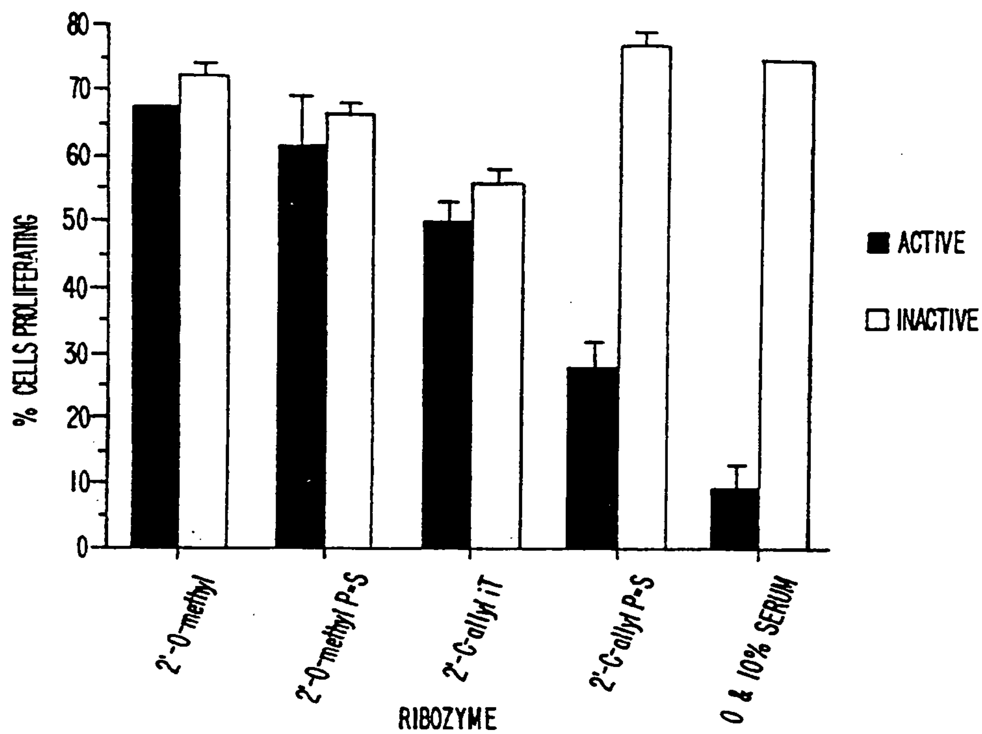
FIG. 8.



% mRNA CLEAVED
0
OLIGONUCLEOTIDE SITE
EXPERIMENT 1
EXPERIMENT 2
2875
2772
2139
2131
2118
1943
1909
1895
1892
1811
1724
1721
1635
1598
1597
1553
1404
1389
1363
1346
1334
1224
1203
1082
1017
936
839
738
634
575
551
549

FIG. 9a

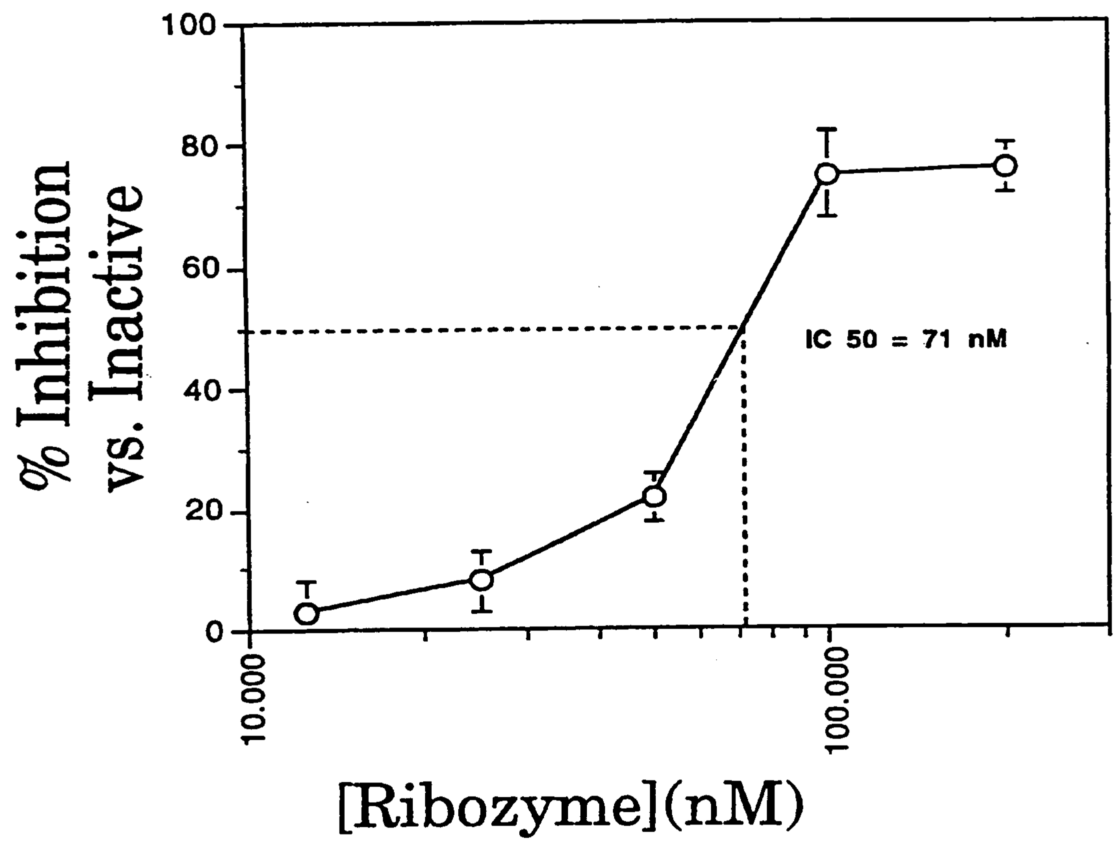

5' ---GGAGAAUU GGAAAAC --- 3'
3' ccucuAA CCuuuug 5'
Uppercase=ribonucleotides
Lower case = 2'-O-methyhucleotides
2'-O-Methyl Ribozyme
5' ---GGAGAAUU GGAAAAC --- 3'
3' c c u c u UA C c u u u g 5'
Uppercase=ribonucleotides
Lower case = 2'-O-methyhucleotides
s=phosphorothioatelinkages
2'-O-Methyl P=S Ribozyme
5'--- GGAGAAUU GGAAAAC ---3'
3'-iT ccucuuA ccuuuug 5'
Uppercase=ribonucleotides
Lower case = 2'-O-methyhucleotides
= 2'-C-Allyl
iT = 3'-3' inverted T
2'-C-Allyl iT Ribozyme
5'--- GGAGAAUU GGAAAAC --- 3'
3' ccucuuA ccuuuug 5'
Uppercase=ribonucleotides
Lower case = 2'-O-methyhucleotides
= 2'-C-Allyl
s=phosphorothioatelinkages
2'-C-Allyl P=S Ribozyme



% CELLS PROLIFERATING
80
70
60
50
40
30
20
10
0
2'-O-methyl
2'-O-methyl P=S
2'-C-allyl iT
2'-C-allyl P=S
0 & 10% SERUM
RIBOZYME
ACTIVE
INACTIVE

FIG. 9b.

FIG. 10.


100
80
60
40
20
0
% Inhibition vs. Inactive
IC 50 = 71 nM
10.000
100.000
[Ribozyme](nM)

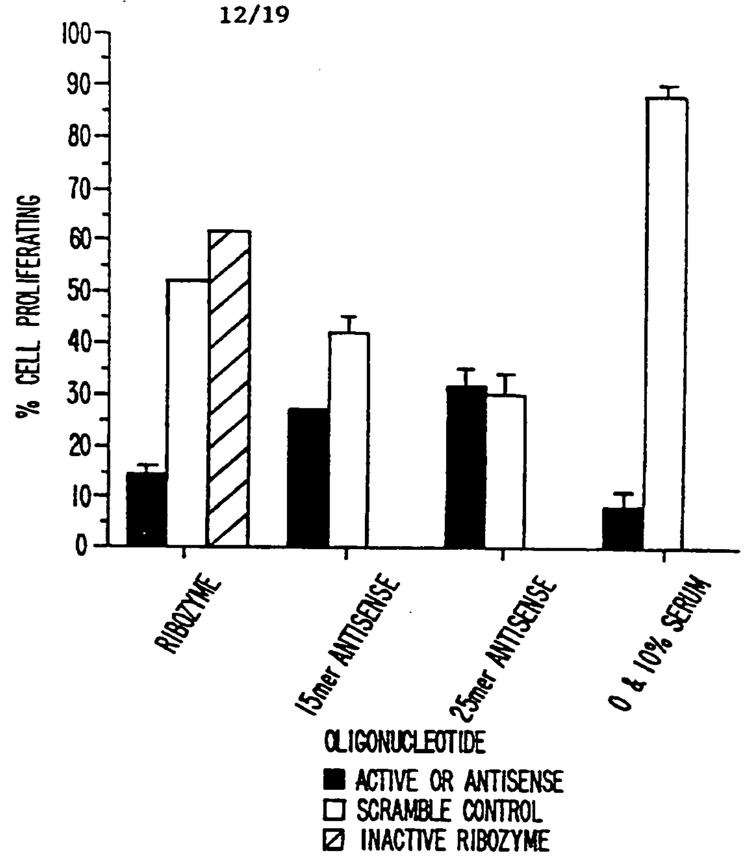

100
90
80
70
60
50
40
30
20
10
0
% CELL PROLIFERATING
RIBOZYME
15mer ANTISENSE
25mer ANTISENSE
0 & 10% SERUM
OLIGONUCLEOTIDE
ACTIVE OR ANTISENSE
SCRAMBLE CONTROL
INACTIVE RIBOZYME

FIG. 11.

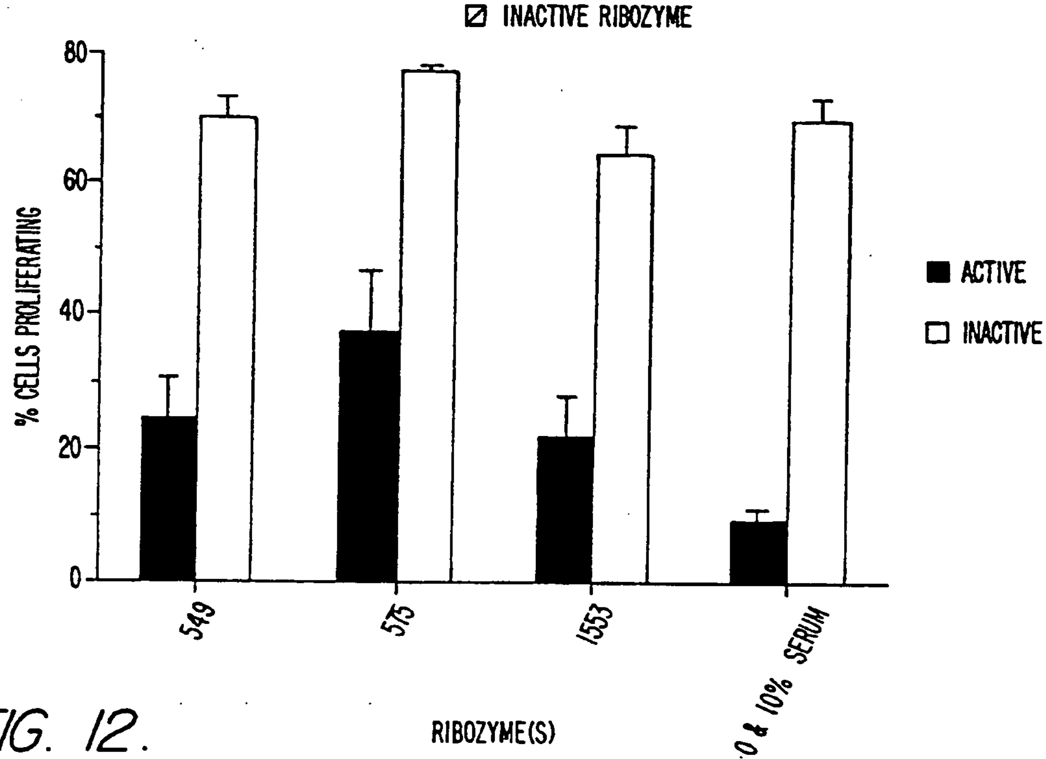
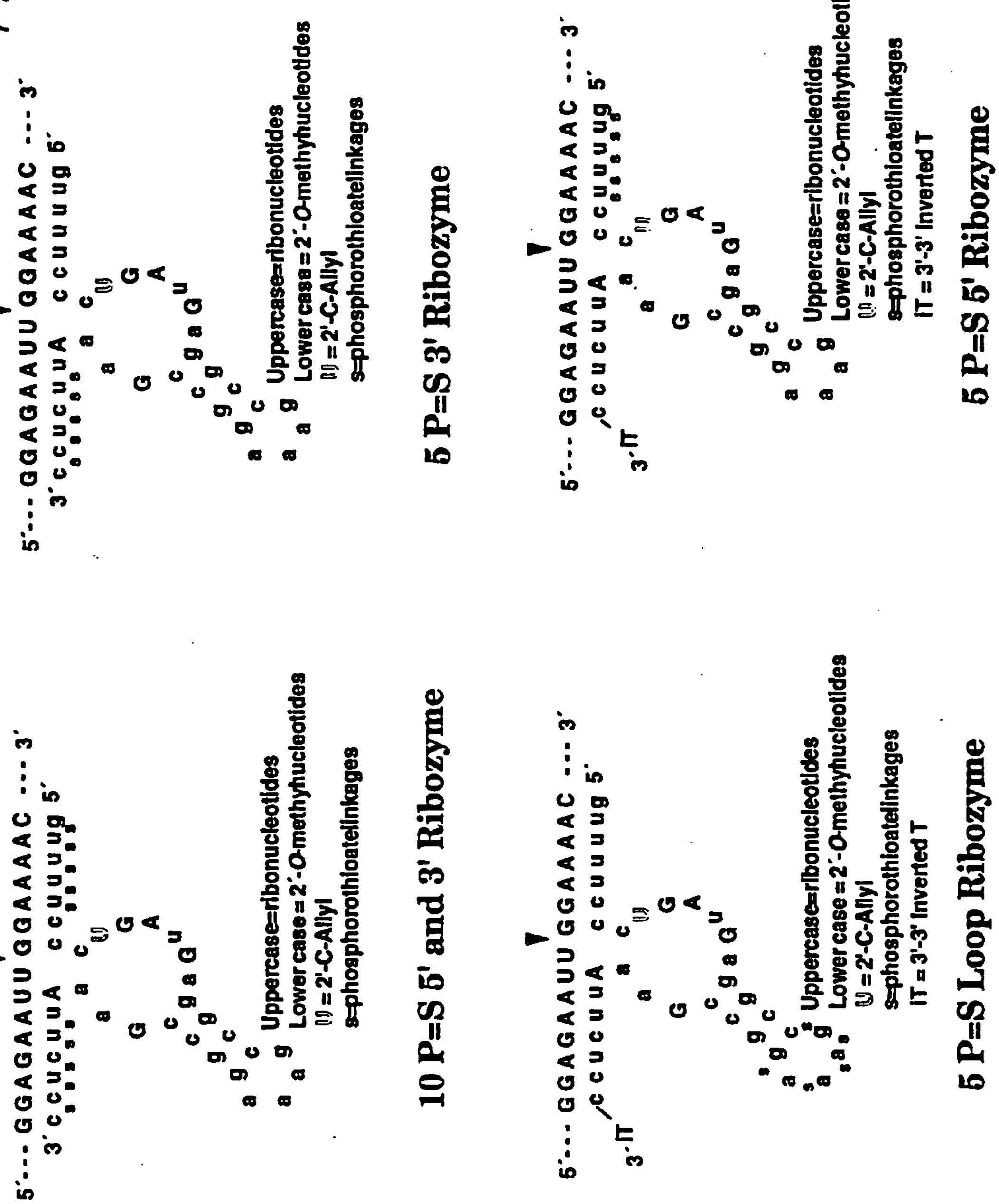

80
60
40
20
0
% CELLS PROLIFERATING
549
575
1553
0 & 10% SERUM
ACTIVE
INACTIVE
RIBOZYME(S)

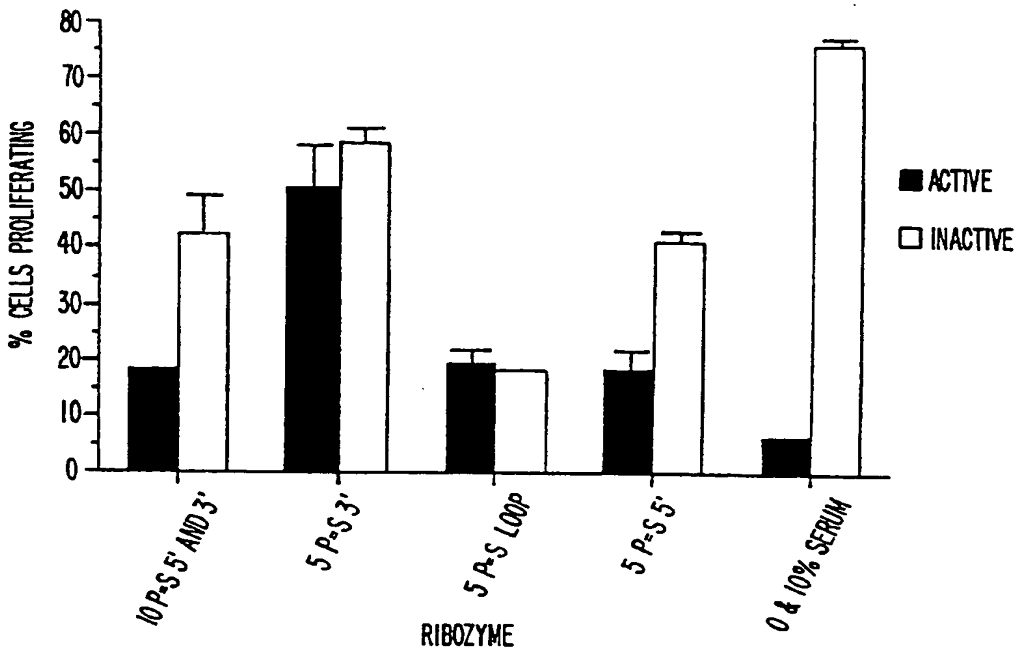
FIG. 12.



5'--- GGAGAAUU GGAAAAC --- 3'
Uppercase=ribonucleotides
Lower case = 2'-O-methylnucleotides
U = 2'-C-Allyl
s=phosphorothioatelinkages
IT = 3'-3' Inverted T
10 P=S 5' and 3' Ribozyme
5 P=S 3' Ribozyme
5 P=S Loop Ribozyme
5 P=S 5' Ribozyme

FIG. 13a.

FIG. 13b.



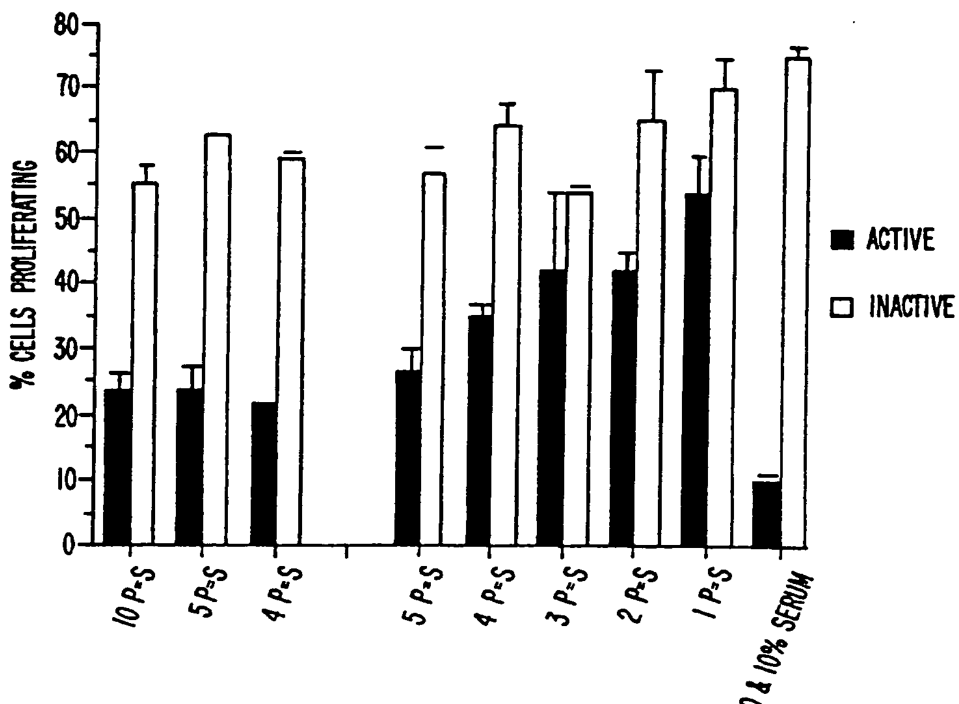
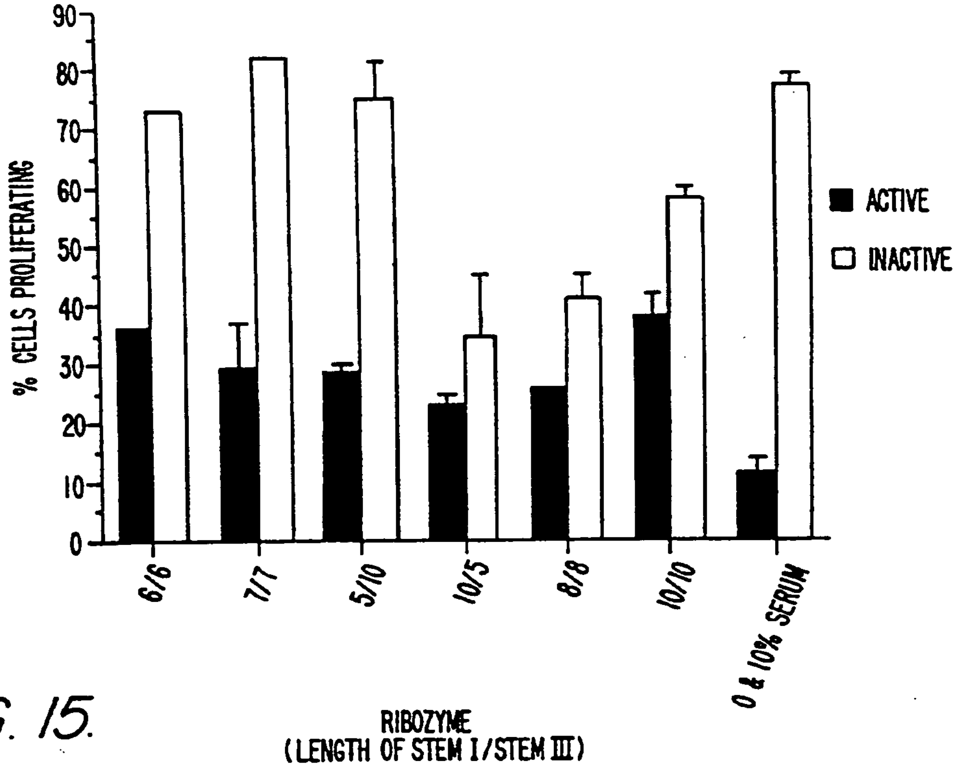
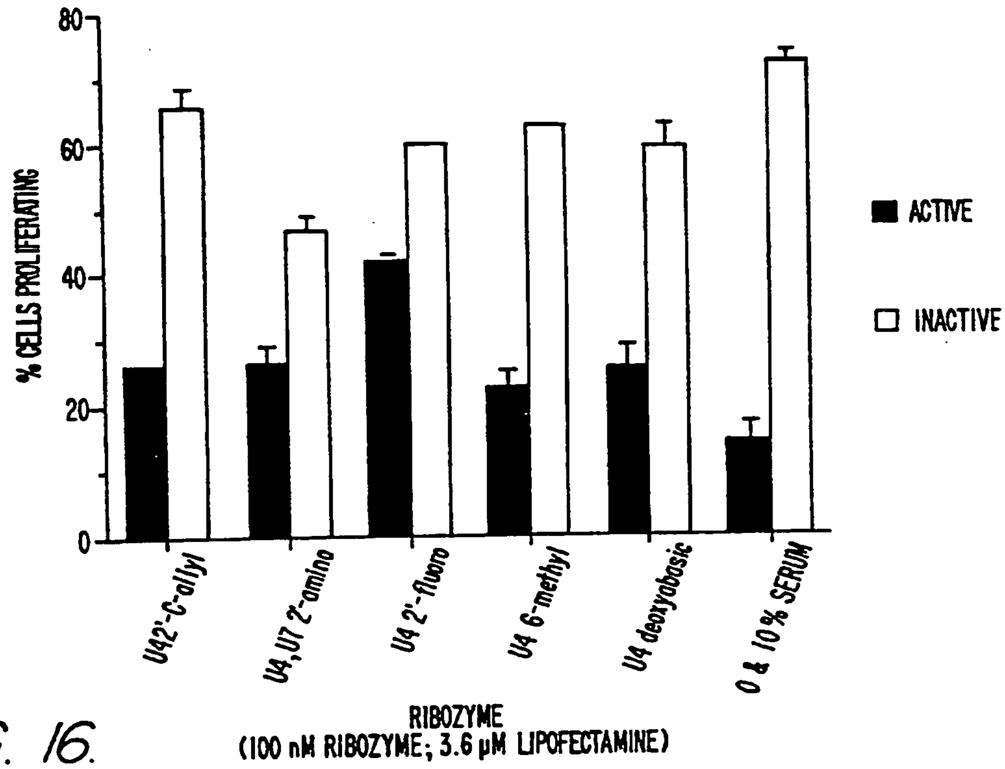


FIG. 14.



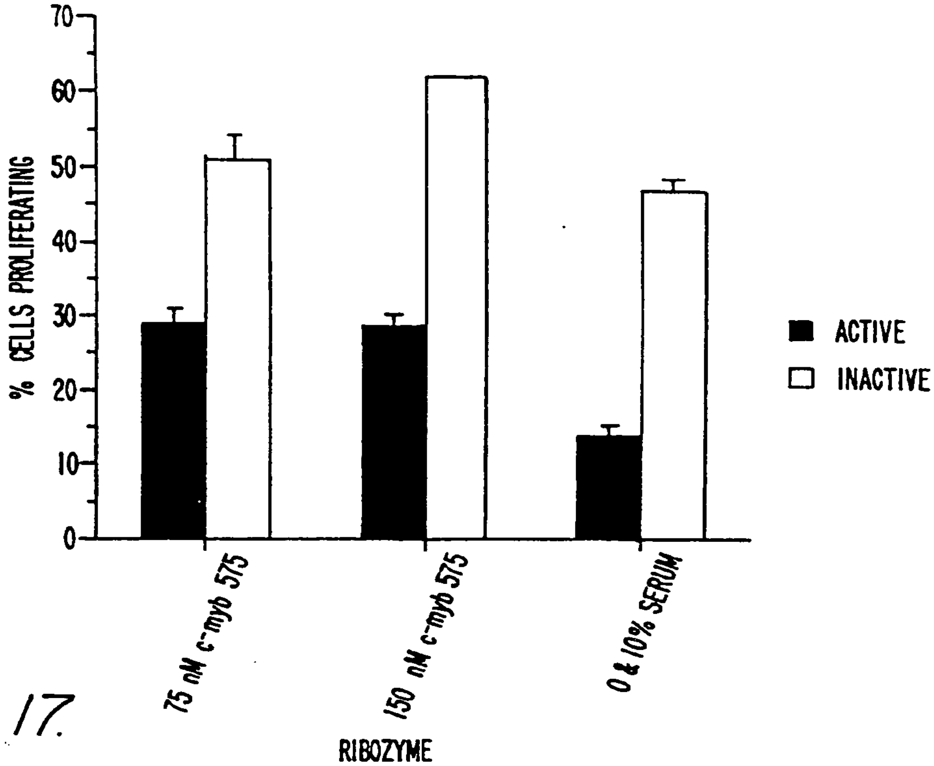
90
80
70
60
50
40
30
20
10
0
% CELLS PROLIFERATING
ACTIVE
INACTIVE
6/6
7/7
5/10
10/5
8/8
10/10
0 & 10% SERUM
RIBOZYME
(LENGTH OF STEM I/STEM III)

FIG. 15.



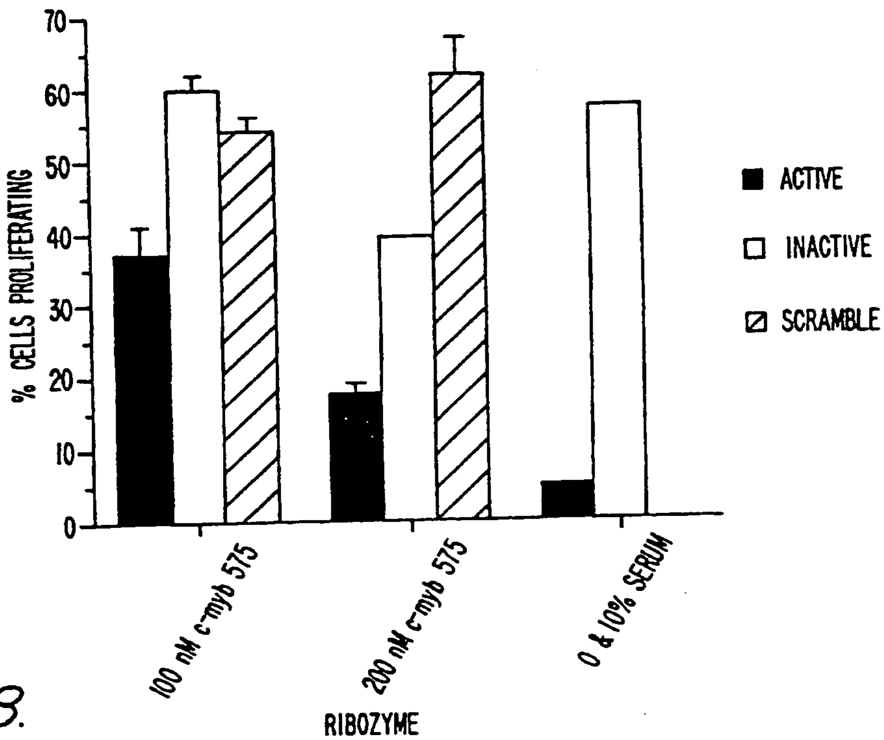
80
60
40
20
0
% CELLS PROLIFERATING
ACTIVE
INACTIVE
U42'-C-allyl
U4,U7 2'-amino
U4 2'-fluoro
U4 6-methyl
U4 deoxyabasic
0 & 10% SERUM
RIBOZYME
(100 nM RIBOZYME; 3.6 μM LIPOFECTAMINE)

FIG. 16.



% CELLS PROLIFERATING
0
10
20
30
40
50
60
70
ACTIVE
INACTIVE
75 nM c-myb 575
150 nM c-myb 575
0 & 10% SERUM
RIBOZYME

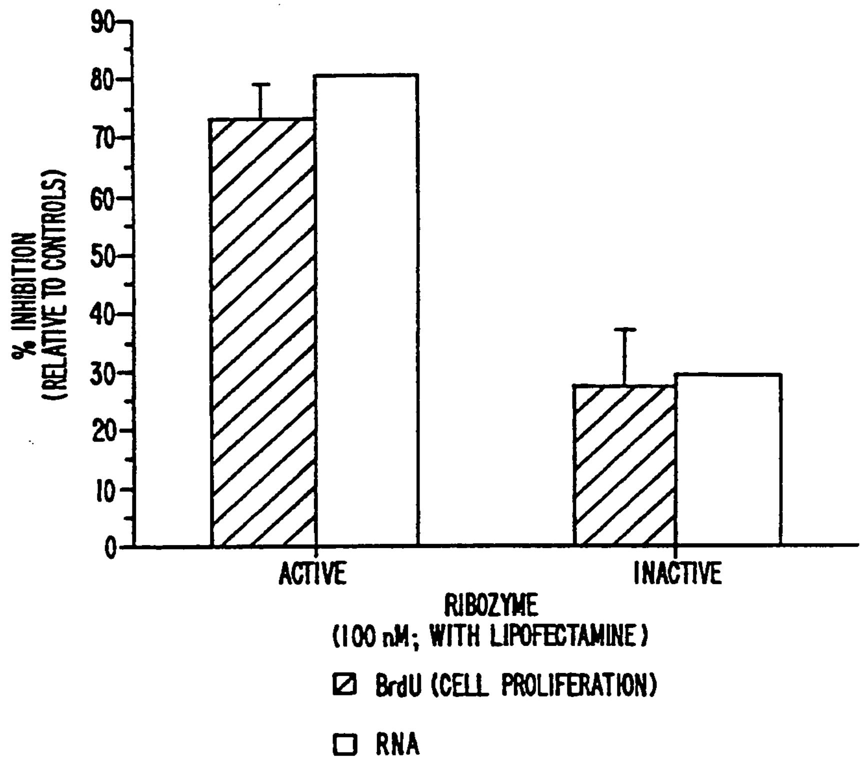
FIG. 17.



% CELLS PROLIFERATING
0
10
20
30
40
50
60
70
ACTIVE
INACTIVE
SCRAMBLE
100 nM c-myb 575
200 nM c-myb 575
0 & 10% SERUM
RIBOZYME

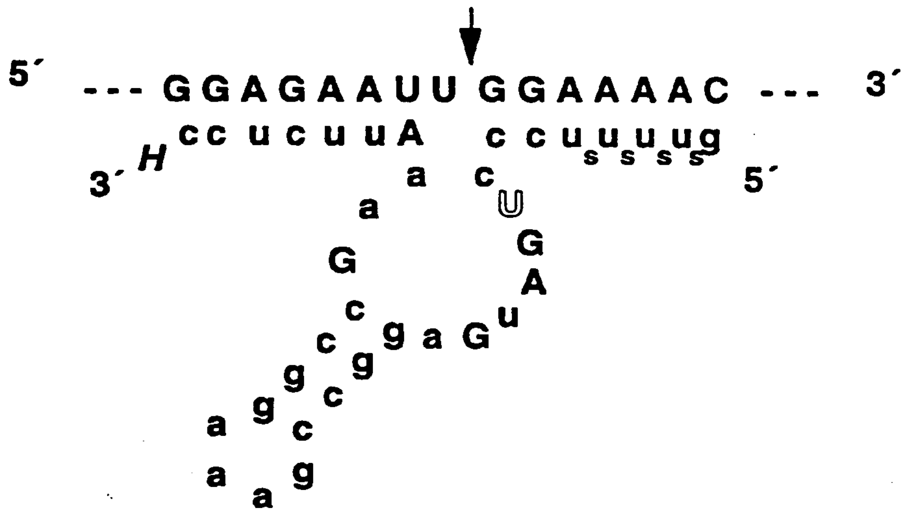
FIG. 18.

FIG. 19.



90
80
70
60
50
40
30
20
10
0
% INHIBITION (RELATIVE TO CONTROLS)
ACTIVE
INACTIVE
RIBOZYME
(100 nM; WITH LIPOFECTAMINE)
BrdU (CELL PROLIFERATION)
RNA

FIG. 20



Uppercase=ribonucleotides

Lower case = 2´-O-methylnucleotides

H = 3´-3´ abasic deoxyribose

U = 2´C-allyl

s=phosphorothioatelinkages

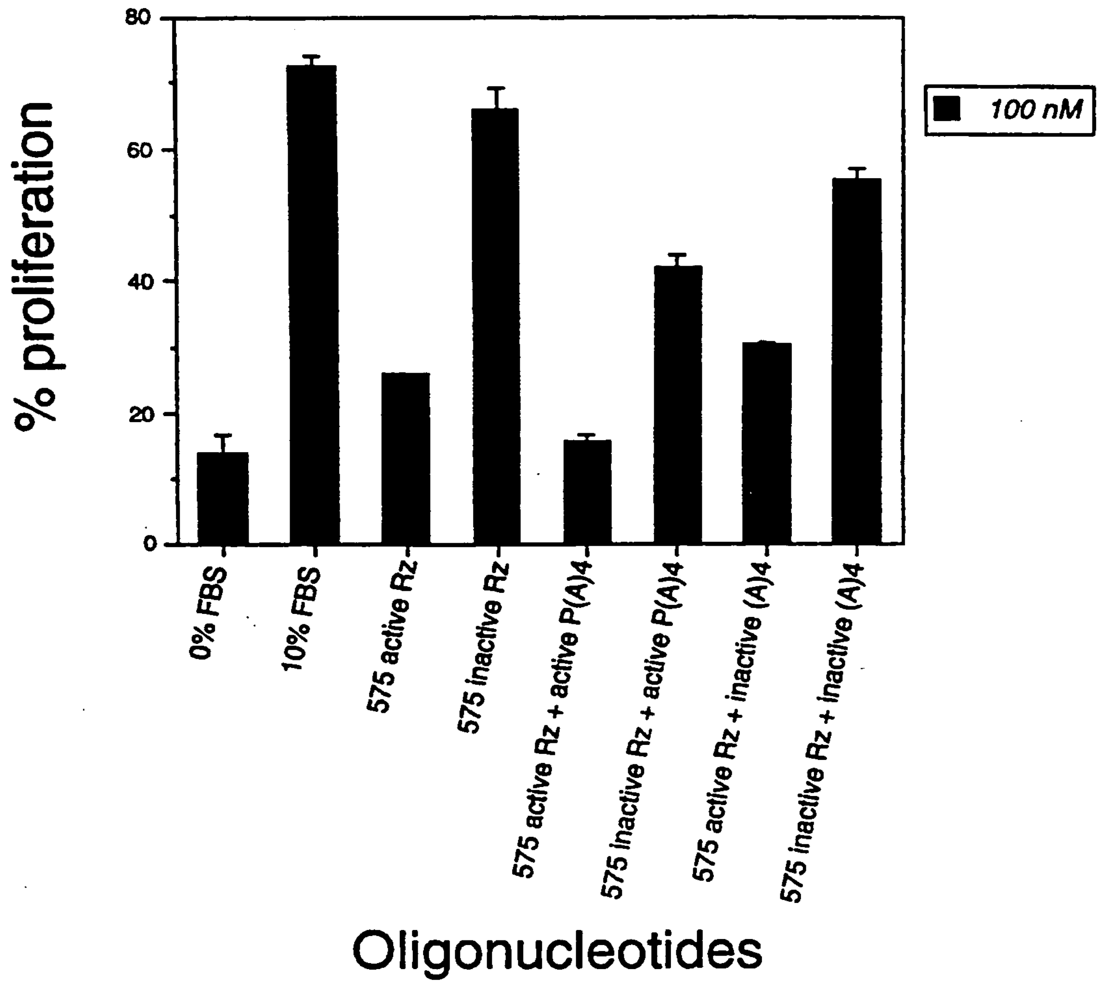

% proliferation
80
60
40
20
0
100 nM
0% FBS
10% FBS
575 active Rz
575 inactive Rz
575 active Rz + active P(A)4
575 inactive Rz + active P(A)4
575 active Rz + inactive (A)4
575 inactive Rz + inactive (A)4
Oligonucleotides

FIG. 21.